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Cranberries: ripe for more cancer research?

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Abstract

Berries have been recognized as a functional food with potential to protect against a variety of health conditions, including some cancers. Cranberry (*Vaccinium macrocarpon*) production and consumption have grown in recent years, warranting further evaluation of potential health benefits. Extracts and isolated constituents from cranberry fruit inhibit growth and proliferation of tumor cells *in vitro*, and recent data from animal studies lend further support to cranberry's reputation as a cancer fighter. Several likely mechanisms of action for cranberry against prostate and other cancers have been identified, including induction of apoptosis and inhibition of events linked to cellular invasion and migration. This article attempts to put into perspective what is known about cranberry's potential chemopreventive properties, what is yet to be determined, and some factors to consider as research moves forward.

Keywords: cranberry; cancer; polyphenol; proanthocyanidin; ursolic acid; tumor; prostate; apoptosis

INTRODUCTION: WHY BERRIES?

Berries are receiving attention for their remarkable health-promoting properties, which are often linked to their high antioxidant content. Berry composition varies widely among species, but most contain a variety of polyphenolic compounds including flavonoids, tannins and stilbenes – all of which are known for antioxidant properties. This antioxidant activity is not limited to radical scavenging, but includes modulation of enzyme activity, gene expression, and signaling pathways associated with oxidative processes and cell proliferation.[1] Most of the data on berries and cancer have come from *in vitro* studies, but protective properties against some cancers are also supported by animal studies. For example, black raspberries (*Rubus occidentalis*), which have been particularly well studied *in vivo*, reduce esophageal, colon, mammary and oral carcinogenesis in rodent models. Clinical trials have demonstrated a reduction in tumorigenesis and associated events in oral cancer and colon cancer patients consuming black raspberry powder.[2] The science supporting black raspberry's anticancer properties is fairly advanced, and provides a goalpost that may be used to guide future research on the chemopreventive effects of other berries. The *in vivo* anticancer activities of most berries have been studied less extensively than those of black raspberry. However, a review of the existing data on some of these fruits, particularly cranberries, suggests that further study of the mechanism and *in vivo* efficacy is justified. The field is ripe for the picking.

CRANBERRY CONSUMPTION IS ON THE RISE

Cranberries (*Vaccinium macrocarpon* Ait., family Ericaceae) are cultivated widely across approximately 40 000 acres in the northern USA and Canada.[3] Relatives of this low-growing, woody perennial vine with short vertical upright branches include the lowbush blueberry (*V. angustifolium*) and bilberry (*V. myrtillus*). Cranberries were used by Native Americans to preserve dried meats and to treat wounds. US cranberry production hit a high of 7 million barrels in 2008 (www.cranberries.org/cranberries/pop_stats_production.html) as a result of growing consumer demand, largely driven by studies showing that cranberry juice can stave off urinary tract infections (UTI). Cranberry products for dietary consumption include juice and other beverages, whole fruit, sweetened dried fruit, sauces, and supplements. The consumption of cranberry juice or supplements to prevent urinary tract infections by *E. coli* bacteria has become common practice, supported by a growing body of scientific evidence.[4,5] As our population ages, it is likely that more individuals will consume these products. In addition to UTI protection, cranberries' known antibacterial properties include inhibition of oral bacteria that cause periodontal diseases[6] and *H. pylori*.[7] Like other *Vaccinium* berries, studies also suggest that the high polyphenol antioxidant content of cranberries[8] may afford some protection against cardiovascular conditions, stroke, and other diseases of aging. Studies relating to these conditions have been reviewed recently and are outside the scope of this article.[9] The current findings on cranberry phytochemicals, their diverse biological activities and their stability and bioavailability have also been comprehensively reviewed.[10] As cranberry consumption increases worldwide, it is important for consumers and scientists alike to understand the potential health benefits cranberry could offer to an aging population, for example whether dietary cranberry offers any protection against various cancers. Cancer is a complex disease involving numerous cellular and molecular processes that could be affected by phytochemicals, as was demonstrated with black raspberry. Despite a wealth of *in vitro* data on anticancer properties, questions remain as to the mechanisms of action of cranberry, the bioavailability of active constituents and their metabolites, and *in vivo* efficacy. The rest of this article attempts to address what is known

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about cranberry composition, possible anticancer mechanisms and data from *in vivo* cancer models, and to offer some perspective on the directions future cranberry–cancer research may take.

CRANBERRY PHYTOCHEMICALS THAT MAY CONTRIBUTE TO CHEMOPREVENTION

The diverse composition of natural health-promoting compounds in the fruit may be one reason why cranberry holds so much promise to limit cancer and other diseases of aging. Cranberry fruit contains a multitude of phytochemicals that have shown antiproliferative activities in tumor cell lines and other properties associated with chemoprevention. Proanthocyanidin oligomers, flavonol and anthocyanin glycosides and ursolic acid and its derivatives are all likely contributors to the observed anticancer properties based on *in vitro* studies. When whole fruit or juice is consumed, these constituents may act in a complementary fashion to limit carcinogenesis. This presence of so many potential bioactive compounds makes it important that extracts used in bioactivity studies are well characterized.

Most of the studies on cranberry's urinary tract health benefits and many cancer-related studies have focused on cranberry proanthocyanidins (PACs) or poly-flavan-3-ols, which are primarily dimers, trimers and larger oligomers of epicatechin. The United States Department of Agriculture (USDA) reports that 100 g cranberry fruit typically contains 180 mg of oligomers with 10 degrees of polymerization (DP) or less, and the content of larger polymers is even higher.[11] PACs are by far the most plentiful flavonoid constituent of cranberry fruit by weight. PACs from *Vaccinium* fruit contain both the more common B-type $4\beta \rightarrow 8$ linkage between epicatechin units and the less common A-type linkage featuring both $4\beta \rightarrow 8$ and $2\beta \rightarrow O \rightarrow 7$ interflavan bonds. The combination of linkages makes cranberry oligomers diverse in three-dimensional structure. We have isolated cranberry proanthocyanidin fractions from whole fruit containing oligomers up to 12 DP. Through fractionation of whole fruit extract, a proanthocyanidin subfraction was obtained which selectively inhibited the proliferation of H460 human large cell lung carcinoma, HT-29 colon adenocarcinoma and K562 chronic myelogenous leukemia cells *in vitro*.[12] Characterization by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) showed that the fraction contained PAC oligomers composed primarily of four to seven epicatechin units with at least one or two A-type linkages between the units.[12] Cranberry PACs have also been the focus of mechanistic anticancer studies, discussed below.

Other flavonoids in the berries are likely to prevent oxidative processes and may play a role in chemoprevention. The major anthocyanins in cranberry are galactosides and arabinosides of cyanidin and peonidin, with a total content of 25–91 mg 100 g^{-1} of fruit, depending on cultivar.[13] The flavonols, with a total content of 20–30 mg 100 g^{-1} fruit, are primarily quercetin glycosides, and myricetin glycosides are present in lesser quantity.[14] *p*-Coumaric acid and benzoic acid – derivatives of these compounds – and various organic acids are also present.

In addition to the polyphenolics, cranberry fruit contains isoprenoids, some of which may contribute to chemoprevention. Ursolic acid – a triterpenoid with established anti-inflammatory and antiproliferative properties – is found in its free form and as the *cis*- and *trans*-*p*-hydroxycinnamoyl esters, which have been observed to inhibit the growth of several tumor cell lines.[14,15] Analysis of fruit from several *Vaccinium* species by liquid chromatography–mass spectrometry (LC-MS) found that *V. macrocarpon* had the highest content of free and esterified ursolic acid among these species.[16] The ursolic acid content in the samples tested ranged from 0.46 to 1.09 g kg^{-1} fresh weight of fruit and the content of its *p*-hydroxycinnamoyl esters was 0.04–0.16 g kg^{-1} fresh weight. By comparison, the total content of free and esterified ursolic acid in *V. vitis-idaea, V. oxycoccus* or *V. angustifolium* was 0.26 g kg^{-1} fresh weight or less. Cranberries contain lutein and other xanthophylls – carotenoids which have been linked to reduced risk of macular degeneration. Iridoids are also present as glycosides in the fruit. The chemopreventive contribution of some of these constituents has yet to be determined.

POSSIBLE MECHANISMS OF ACTION IN TUMOR CELLS

The phytochemicals in cranberries may act in a complementary or synergistic manner in chemoprevention.[1] Each of the cranberry constituents discussed above (quercetin, proanthocyanidins, ursolic acid and anthocyanins) has been reported independently to possess antiproliferative or anti-inflammatory activities; these have been reviewed.[14,17] Possible mechanisms of action against carcinogenesis that are supported by *in vitro* evidence include induction of cellular apoptosis, decreased invasion and metastasis as a result of matrix metalloproteinase inhibition, decreased ornithine decarboxylase expression and activity, antioxidant activities that may reduce oxidative stress, and inhibition of inflammatory processes including cyclooxygenase-2 (COX-2) activity.[14,17]

One of the earliest studies to address the mechanism of action of cranberry and other *Vaccinium* berry polyphenols found that ornithine decarboxylase – a key enzyme in the biosynthesis and metabolism of polyamines involved in cell proliferation – may be involved.[18] Over-expression of these enzymes is observed in models of cancer and can be induced by pro-inflammatory agents. A cranberry fruit flavonoid fraction was observed to inhibit ornithine decarboxylase (ODC) activity in a murine epidermal cell line (ME-308), as determined by an assay measuring conversion of substrate.[19] Preliminary studies also suggest that cranberry polyphenolics inhibit expression of ODC induced by lipopolysaccharides (LPS) in H-ras-transformed mouse fibroblasts.[20] Berry constituents, particularly anthocyanins, also have been observed to limit angiogenesis,[21] though these activities have not yet been demonstrated using cranberry alone.

The cytotoxicity of cranberry constituents towards numerous tumor cell lines has been reported using standard 48 h growth inhibition assays.[12,15,16] The constituents vary in their degree of cytotoxicity. Whereas ursolic acid is cytotoxic towards most tumor cells at concentrations <10 µmol L^{-1}, proanthocyanidins are more selective, and generally show significant cytotoxicity only at concentrations >10 µmol L^{-1}. At concentrations <10 µmol L^{-1}, however, cranberry proanthocyanidins significantly inhibited colon tumor colony formation over a 2-week period.[22] The observed cytotoxicity of cranberry constituents may be due in part to induction of apoptosis. Several studies report on induction of apoptosis by cranberry polyphenolics or triterpenoids in cancer cell lines, including the MDA-MB-435 and MCF-7 breast carcinoma cell lines,[23,24] colon carcinoma tumor cell lines HCT116 and HT-29,[22] U87 glioblastoma cells,[25] oral squamous cell carcinomas CAL27 and SCC25,[26] and SKOV-3 ovarian cancer cells.[27] Inhibition of acid-induced proliferation of SEG-1 esophageal cells by cranberry

PAC was accompanied by cell cycle arrest at G1 checkpoint and morphological changes characteristic of apoptosis.[28]

Apoptosis is a complex process involving multiple cellular signaling pathways; therefore further studies are needed to pinpoint which apoptosis pathways may be activated by cranberry constituents in a given cell line.

CRANBERRY AND PROSTATE CANCER: MULTIPLE MECHANISMS ARE POSSIBLE

Cranberry extracts have demonstrated antiproliferative activities against prostate tumor cell lines in several *in vitro* studies.[12,15,25,29] Inhibition of prostate tumor cell growth has been observed with whole cranberry extract, cranberry powder, polyphenolic extracts, proanthocyanidin fractions isolated from the fruit, and ursolic acid esters isolated from the fruit. Cranberry PACs reduced DU145 cell viability by 50% over 24 h at concentrations as low as 10 $\mu g\ mL^{-1}$.[30] Recent studies using androgen-independent DU145 prostate carcinoma cells point to some possible mechanisms of action by cranberry and its constituents. One mechanism by which cells keep their population in check is apoptosis, and cranberry phytochemicals have been observed to induce apoptosis in a variety of tumor cell lines, as mentioned above. Apoptosis can occur by multiple pathways, and some of these were investigated by us in DU145 cells.[30] Treatment of DU145 cells with crude cranberry extract prepared from whole fruit for 24 h at 100 $\mu g\ mL^{-1}$ increased apoptosis twofold, based on DNA fragmentation. A significant increase was also observed at a dose of 10 $\mu g\ mL^{-1}$. Protein expression measurement showed that treatment at both concentrations increased release of cytochrome C, required for apoptosome assembly, from the mitochondria into the cytosol as early as 6 h after treatment, activating caspase-9. Upregulation of Par-4 and Bax, and proteolytic cleavage of Bid to tBid, were also observed – events that could be instrumental in the release of cytochrome C. Both PAC and flavonol-enriched fractions of the fruit activated caspase-8 and caspase-9 6–12 h after treatment; both of these events are likely to contribute to increased apoptogenic activity.[30] We have observed that cranberry proanthocyanidins and ursolic acid also induce apoptosis in HCT-116 and HT-29 colon carcinoma cells,[22] and are presently elucidating the mechanisms of action in these cell lines. A recent study also reported that cranberry PACs downregulated expression of inhibitors of apoptosis proteins (IAPs) and other anti-apoptotic molecules in H460 lung cancer cell line,[31] consistent with earlier studies that found proliferation of this cell line reduced by treatment with cranberry PAC fractions.[12]

Another potential route to chemoprevention involves blocking cell invasion and migration – events associated with metastasis and a critical point in the progression of cancer originating in the prostate. Androgen-independent cancerous cells are particularly able to do this, enabled by the matrix metalloproteinases (MMPs), which are gelatinases that degrade the extracellular matrix, promoting metastasis.[32] Treatment of DU145 cells with proanthocyanidins isolated from whole cranberry fruit at 25 $\mu g\ mL^{-1}$ reduced the activity of matrix metalloproteinase-2 and -9 significantly in as little as 1 h, independent of the cytotoxicity which occurred after 6 h treatment.[33] Similar behavior has been reported with grape seed PACs in this cell line. The effect of cranberry PACs on prostate cells was accompanied by increased expression of TIMP-2 (tissue inhibitor of matrix metalloproteinase) and a decrease in expression of the MMP inducer EMMPRIN (extracellular MMP inducer). PAC treatment also increased phosphorylation of p38, ERK1 and ERK2, all signaling proteins associated with the mitogen-activated protein kinase (MAPK) pathway, as well as expression of signaling proteins in the phosphatidylinositol-3-kinase (PI-3) pathway.[33] The proanthocyanidin fractions used in this study were isolated from whole cranberry fruit. MALDI-TOF MS analysis found the fractions were composed primarily of dimers through octamers of epicatechin, typically containing one A-type linkage in each oligomer.

Although no feeding studies employing an animal model of prostate cancer have yet appeared in the literature, a study examined the effects of cranberry treatment by injection on the development of explant prostate tumors in female nude mice.[25] Mice were given intraperitoneal injections at several time points over 3 weeks, and the volume of developing tumors was monitored over the next 100 days. The cranberry-treated mice developed tumors much more slowly than control, reaching a significantly lower maximum volume 30 days later, after which the tumors regressed in size. Similarly, cranberry treatment slowed the growth of HT29 colon and U87 glioma explants. This was the first study to demonstrate *in vivo* efficacy of cranberry against tumorigenesis.[25]

BIOAVAILABILITY OF CRANBERRY CONSTITUENTS: WHAT DO WE KNOW?

The question of bioavailability of cranberry constituents is a critically important one, to be considered as research progresses from the *in vitro* stages to animal or clinical studies. Flavonoids are extensively conjugated and metabolized *in vivo*, and clinical studies show that only a small percentage of the anthocyanins or flavonols consumed orally appear intact in the urine, and even less in plasma.[34] A recent clinical study of subjects consuming a high berry polyphenol diet for 8 weeks detected some intact quercetin in the plasma and urine, but most of the flavonoid skeletons were broken down to phenolic metabolites such as homovanillic acid, 3-hydroxyphenylacetic acid and 3-(3-hydroxyphenyl)propionic acid.[35] While cranberries were not part of the berry diet in that clinical study, they contain similar polyphenols. A Japanese study examined the urine of human subjects consuming a single 0.200 L dose of cranberry juice by LC-MS, and found several intact anthocyanins, including peonidin-3-galactoside, present 24 h later[36] (Ohnishi *et al*., 2006). Data from studies with rodents also suggest that a small percentage of flavonoids remains intact, though most are broken down. A study of bladder cancer in mice given cranberry juice concentrate by gavage detected quercetin and methyl quercetin in the urine 4 h after treatment.[34] A study of urinary metabolites in rats fed cranberry powder found the metabolites were primarily 4-hydroxyphenylacetic acid, hippuric acid, and 3-hydroxyphenylacetic acid. 4-hydroxycinnamic acid, which is a major constituent of the fruit, was also detected in significant quantity.[37] The fate of oligomeric proanthocyanidins in the human body is a topic of ongoing investigation. Although both cranberry proanthocyanidins and urine of subjects who consume cranberry show antibacterial adhesion properties, intact PACs are not detected in the urine, nor have the active metabolites been identified. Most studies suggest that oligomers larger than dimers are not absorbed appreciably. They may be transformed by colonic bacteria to their bioactive metabolites,[37] which have yet to be fully characterized, but are likely to include substituted phenolic acids. A recent comprehensive review of the existing science on berry flavonoid metabolism emphasizes the importance of future

research addressing the relationship between polyphenols and the colonic microflora, and the nature and activities of catabolites.[38]

Even less is known about the metabolic fate of ursolic acid, a compound which lacks a chromophore and is more difficult to detect than flavonoids and other phenolics. While a great deal of *in vitro* data demonstrates ursolic acid's anticancer activities, its bioavailability has not been established. One of the first studies to address ursolic acid's *in vivo* anticancer activity appeared in 2010. The effects of ursolic acid administered orally were evaluated in a mouse model of postmenopausal breast cancer.[39] Ovariectomized C57BL/6 mice received a diet supplemented with ursolic acid at doses of 0.05%, 0.10% or 0.25% by weight for a period of 8 weeks. After 3 weeks, mice were injected with syngeneic MMTV-Wnt-1 mammary tumors. Appearance of tumors and their volume and weight were monitored for the next 5 weeks. The most positive results were observed in the 0.10% ursolic acid group, in which tumor appearance was delayed, the size and weight of tumors was significantly lower than the control group, and 40% of mice showed no evidence of tumors at the end of the study. Tissues were not analyzed for ursolic acid or its metabolites, but effects on the tumor cells *in vivo* were consistent with those reported previously *in vitro*: induction of apoptosis, increased caspase-3 expression, decreased phosphorylation of protein kinases Akt and MAPK. The study suggests that dietary ursolic acid may be chemopreventive,[39] and further study of its metabolites and mechanisms is warranted. As Pappas and Schaich point out, the health-promoting capacity of cranberry phytochemicals of cranberries other than proanthocyanidins has been largely overlooked and should be considered further.[10]

ANIMAL CANCER MODEL STUDIES FIND CRANBERRY REDUCES TUMOR INCIDENCE AND SIZE

The *in vitro* data on anticancer activities of cranberry constituents support a role for flavonoids, proanthocyanidins and ursolic acid derivatives in chemoprevention. The question of whether these compounds or their metabolites are bioavailable to the tissues affected by various cancers in sufficient quantity to elicit protective effects can only be answered by considering cranberry's activity *in vivo*. Recent animal model studies suggest that cranberry treatment may be effective in reducing the incidence and severity of some cancers. The first reported study in mice used tumor explants to model cancers of the prostate, colon and nervous system. As noted above, administration of cranberry extracts by injection over a period of several months decreased the rate of growth and size of tumors in these models.[25] The extract used in the treatments was described as a proanthocyanidin-rich fraction from whole cranberries. A fraction from cranberry presscake also inhibited growth of the glioblastoma.[25] Cranberry press cake has not been fully characterized in the literature, but is likely to contain larger oligomeric proanthocyanidins as well as nonpolar berry constituents such as ursolic acid that do not appear in significant quantities in cranberry juice. It has been shown that the content of ursolic acid and derivatives is much higher in whole cranberry fruit and products than in the juice or products derived from the juice.[16]

A second mouse study in 2008 used high-molecular-weight, nondialyzable material (NDM), a cranberry juice preparation previously studied for its antibacterial properties. The effect of cranberry NDM on development of lymphoma was investigated in immune-competent, syngeneic mice.[40] The two NDM extracts used in this study had approximate molecular mass ranges of >12K or 12–30K based on preparation methods, and were obtained from the juice by dialysis and ultrafiltration. While cranberry NDM has not been fully characterized, it is thought to be at least partly composed of proanthocyanidin oligomers. Both extracts decreased the proliferation of Rev-2-T-6 lymphoma cells *in vitro*, and reduced invasion of these cells through Matrigel. Only NDM of 12–30K range was tested in mice, due to higher toxicity of the other fraction. Mice received intraperitoneal injections at a nontoxic dose every other day for 2 weeks and were monitored for up to 100 days after treatment. While 80% of the control group developed palpable tumors within 3 weeks, the cranberry-treated group showed no tumor development throughout the study. Interestingly, the NDM (12–30K) treated mice generated antibodies against the Rev-2-T-6 cells, suggesting that NDM increased the mouse immune response against the lymphoma, a response which was directed against mouse mammary tumor virus (MMTV) antigens.[40]

Direct injection of cranberry constituents in mice appears to provide some protection against the development of certain cancers, but what about dietary consumption of cranberry? The first study to address this question examined the effect of cranberry juice concentrate on bladder cancer in rats.[34] Female Fischer 344 rats were induced with a nitrosamine over 8 weeks to induce tumor formation. The cranberry treatment groups then received dosages of either 1.0 or 0.5 mL per rat per day cranberry juice concentrate via gavage for 6 months. At the higher dose, a 31% reduction in bladder tumor weight and a 38% reduction in number of cancerous lesions occurred compared to control. The authors also note that quercetin and methylquercetin were detected in the bladder 12 h after gavage.[34] The authors did not determine whether quercetin was the active constituent. The study suggests that cranberry given orally results in metabolites present in sufficient quantity to inhibit tumorigenesis.

CONSIDERATIONS FOR FUTURE RESEARCH

The existing animal studies are promising, but more work will be needed to determine whether dietary cranberry protects against some of the most common cancers, e.g. colon, prostate and breast cancers. Strong *in vitro* data in colon, prostate and breast carcinoma cells support proceeding on to animal studies, using well-designed feeding trials to determine whether cranberry constituents or metabolites are bioavailable to tissues in quantities sufficient to inhibit tumorigenesis. Ideally, such studies would be carried out with freeze-dried whole fruit powder, as was used in black raspberry–colon cancer studies.[2] A diet prepared with minimal processing of the fruit is likely to contain the complete profile of berry constituents, which is important because existing studies suggest that cranberry phytochemicals may act in a complementary or synergistic way in chemoprevention.[29] Use of high temperatures in processing the fruit is likely to reduce the anthocyanin content, which would negatively impact antioxidant quality. Additionally, when compared to cranberry juice, the whole fruit has a higher content of ursolic acid and larger proanthocyanidin oligomers – two constituents which have shown antiproliferative properties *in vitro*. Given the lack of clear data on metabolites, tissue analysis to determine the identities of the active metabolites of these compounds *in vivo* would be useful. Thorough characterization of cranberry extracts or fractions is recommended for all studies, especially those addressing mechanisms of action and the effects of whole fruit *versus* individual constituents. Structure–activity studies will help

scientists apply what we learn about cranberries to other fruits. As research on berries and cancer moves forward, the future may be berry bright indeed.

REFERENCES

1 Seeram NP, Berries, in *Nutritional Oncology* (2nd edn), ed. by Heber D. Academic Press/Elsevier, New York, pp. 615–628 (2006).
2 Stoner GD and Seeram NP (eds), *Berries and Cancer Prevention*. Springer, New York (2011).
3 Cranberry Institute. [Online]. Available: http://www.cranberryinstitute.org/about_cranberry.htm [12 August 2011].
4 Howell A, Cranberry proanthocyanidins and the maintenance of urinary tract health. *Crit Rev Food Sci Nutr* **42S**:273–278 (2002).
5 Jepson RG and Craig JC, A systematic review of the evidence for cranberries and blueberries in UTI prevention. *Mol Nutr Food Res* **51**:738–745 (2007).
6 Bodet C, Grenier D, Chandad F, Ofek I, Steinberg D and Weiss EI, Potential oral health benefits of cranberry. *Crit Rev Food Sci Nutr* **48**:672–680 (2008).
7 Burger O, Ofek I, Tabak M, Weiss EI, Sharon N and Neeman I, A high molecular mass constituent of cranberry juice inhibits *Helicobacter pylori* adhesion to human gastric mucus. *FEMS Immunol Med Microbiol* **29**:295–301 (2000).
8 Vinson JA, Su X, Zubik L and Bose P, Phenol antioxidant quantity and quality in foods: fruits. *J Agric Food Chem* **49**:5315–5321 (2001).
9 Neto CC, Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. *Mol Nutr Food Res* **51**:652–664 (2007).
10 Pappas E and Schaich KM, Phytochemicals of cranberries and cranberry products: characterization, potential health effects, and processing stability. *Crit Rev Food Sci Nutr* **49**:741–781 (2009).
11 USDA Database for the Proanthocyanidin Content of Selected Foods (August 2004). [Online]. Available: http://www.ars.usda.gov/Services/docs.htm?docid=5843 [12 August 2011].
12 Neto CC, Krueger CG, Lamoureaux TL, Kondo M, Vaisberg AJ, Hurta RAR, *et al*, MALDI-TOF MS characterization of proanthocyanidins from cranberry fruit (*Vaccinium macrocarpon*) that inhibit tumor cell growth and matrix metalloproteinase expression *in vitro*. *J Sci Food Agric* **86**:18–25 (2006).
13 Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE and Prior RL, Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J Agric Food Chem* **54**:4069–4075 (2006).
14 Neto CC, Cranberry and its phytochemicals: a review of *in vitro* anticancer studies. *J Nutr* **137**:186S–193S (2007).
15 Murphy BT, MacKinnon SL, Yan X, Hammond GB, Vaisberg AJ and Neto CC, Identification of triterpene hydroxycinnamates with *in vitro* antitumor activity from whole cranberry fruit (*Vaccinium macrocarpon*). *J Agric Food Chem* **51**:3541–3545 (2003).
16 Kondo M, MacKinnon SL, Craft CC, Matchett MD, Hurta RAR and Neto CC, Ursolic acid and its esters: occurrence in cranberries and other *Vaccinium* fruit and effects on matrix metalloproteinase activity in DU145 prostate tumor cells. *J Sci Food Agric* **91**:789–796 (2011).
17 Neto CC, Amoroso JW and Liberty AM, Anticancer activities of cranberry phytochemicals: an update. *Mol Nutr Food Res* **52**:18–27 (2008).
18 Bomser J, Madhavi DL, Singletary K and Smith MAL, *In vitro* anticancer activity of fruit extracts from *Vaccinium* species. *Planta Med* **62**:212–216 (1996).
19 Kandil FE, Smith MAL, Rogers RB, Pépin MF, Song LL, Pezzuto JM, *et al*, Composition of a chemopreventive proanthocyanidin-rich fraction from cranberry fruits responsible for the inhibition of TPA-induced ODC activity. *J Agric Food Chem* **50**:1063–1069 (2002).
20 Matchett MD, Compton KA, Kondo M, Neto CC and Hurta RAR, Lipopolysaccharide, cranberry flavonoids and regulation of ornithine decarboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT) expression in H-ras transformed cells. *FASEB J*, **19**:A825 (2005).
21 Roy S, Khanna S, Alessio HM, Vider J, Bagchi D, Bagchi M, *et al*, Antiangiogenic property of edible berries. *Free Radic Res* **36**:1023–1031 (2002).
22 Liberty AM, Amoroso JW, Hart PE and Neto CC, Cranberry PACs and triterpenoids: anti-cancer activities in colon tumor cell lines. *Acta Hortic* **841**:61–66 (2009).
23 Ferguson P, Kurowska E, Freeman DJ, Chambers AF and Koropatnick DJ, A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. *J Nutr* **134**:1529–1535 (2004).
24 Sun J and Liu RH, Cranberry phytochemical extracts induce cell cycle arrest and apoptosis in human MCF-7 breast cancer cells. *Cancer Lett* **241**:124–134 (2006).
25 Ferguson PJ, Kurowska EM, Freeman DJ, Chambers AF and Koropatnick DJ, *In vivo* inhibition of growth of human tumor lines by flavonoid fractions from cranberry extract. *Nutr Cancer* **56**:86–94 (2006).
26 Chatelain K, Phippen S, McCabe J, Teeters CA, O'Malley S and Kingsley K, Cranberry and grape seed extracts inhibit the proliferative phenotype of oral squamous cell carcinomas. *Evid Based Complement Alternat Med* (2011). ID 467691 (2011).
27 Singh A, Singh R, Kim K, Satyan K, Nussbaum R, Brard L, *et al*, Cranberry proanthocyanidins are cytotoxic to human cancer cells and sensitize platinum-resistant ovarian cancer cells to paraplatin. *Phytother Res* **23**:1066–1074 (2009).
28 Kresty LA, Howell AB and Baird M, Cranberry proanthocyanidins induce apoptosis and inhibit acid-induced proliferation of human esophageal adenocarcinoma cells. *J Agric Food Chem* **56**:676–680 (2008).
29 Seeram NP, Adams LS, Hardy ML and Heber D, Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *J Agric Food Chem* **52**:2512–2517 (2004).
30 MacLean MA, Scott BE, Deziel BA, Liberty AM, Nunnelley M, Gottschall-Pass KT, *et al*, North American cranberry (*Vaccinium macrocarpon*) stimulates apoptotic pathways in DU145 human prostate cancer cells *in vitro*. *Nutr Cancer* **63**:109–120 (2011).
31 Kresty L, Howell A and Baird M, Cranberry proanthocyanidins mediate growth arrest of lung cancer cells through modulation of gene expression and rapid induction of apoptosis. *Molecules* **16**:2375–2390 (2011).
32 Yousif R, Herzog M, Che M, Upadhyay J, Shekarriz B, Bhagut S, *et al*, Matrix metalloproteinase activity, bone matrix turnover, and tumor cell proliferation in prostate cancer bone metastasis. *J Natl Cancer Inst* **94**:17–25 (2002).
33 Déziel B, Patel K, Neto C, Gottschall-Pass K and Hurta R, Proanthocyanidins from the American cranberry (*Vaccinium macrocarpon*) inhibit matrix metalloproteinase-2 and matrix metalloproteinase-9 activity in human prostate cancer cells via alterations in multiple cellular signalling pathways. *J Cell Biochem* **111**:742–754 (2010).
34 Prasain JK, Jones K, Moore R, Barnes S, Leahy M, Roderick R, *et al*, Effect of cranberry juice concentrate on chemically-induced urinary bladder cancers. *Oncol Rep* **19**:1565–1570 (2008).
35 Koli R, Erlund I, Jula A, Marniemi J, Mattila P and Alfthan G, Bioavailability of various polyphenols from a diet containing moderate amounts of berries. *J Agric Food Chem* **58**:3927–3932 (2010).
36 Ohnishi R, Ito H, Kasajima N, Kaneda M, Kariyama R, Kumon H, *et al*, Urinary excretion of anthocyanins in humans after cranberry juice ingestion. *Biosci Biotechnol Biochem* **70**:1681–1687 (2006).
37 Prior RL, Rogers TR, Khanal RC, Wilkes SE, Wu X and Howard LR, Urinary excretion of phenolic acids in rats fed cranberry. *J Agric Food Chem* **58**:3940–3949 (2010).
38 Del Rio D, Borges G and Crozier A, Berry flavonoids and phenolics: bioavailability and evidence of protective effects. *Br J Nutr* **104**:S67–90 (2010).
39 DeAngel RE, Smith SM, Glickman RD, Perkins SN and Hursting SD, Antitumor effects of ursolic acid in a mouse model of postmenopausal breast cancer. *Nutr Cancer* **62**:1074–1086 (2010).
40 Hochman N, Houri-Haddad Y, Koblinski J, Wahl L, Roniger M, Bar-Sinai A, *et al*, Cranberry juice constituents impair lymphoma growth and augment the generation of antilymphoma antibodies in syngeneic mice. *Nutr Cancer* **60**:511–517 (2008).

