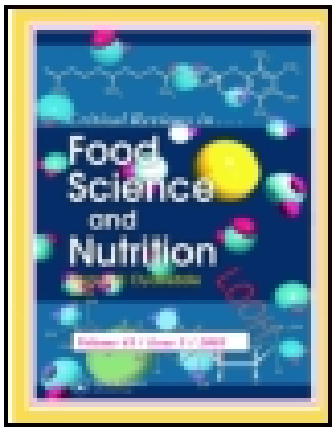

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Potential Benefits of Edible Berries in the Management of Aerodigestive and Gastrointestinal Tract Cancers: Preclinical and Clinical Evidence

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Potential Benefits of Edible Berries in the Management of Aerodigestive and Gastrointestinal Tract Cancers: Preclinical and Clinical Evidence

Running title: Berries and digestive tract cancers

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ABBREVIATIONS

ACE = adenocarcinoma of the esophagus;

ACF = aberrant crypt foci;

Ada = adenosine deaminase;

AOM = azoxymethane;

APC^{Min} = adenomatous polyposis coli (Min);

COX-2 = cyclooxygenase-2;

CRC = colorectal cancer;

DDS = dextran sodium sulfate;

DENA = diethylnitrosamine;

DMBA = 7,12-dimethylbenz(a)anthracene;

DNMT = DNA methyl transferase;

E-cadherin = epithelial-cadherin;

ERK = extracellular signal-regulated kinase;

FAP = familial adenomatous polyposis

GIT = gastrointestinal tract;

GM-CSF = granulocyte macrophage-colony stimulating factor;

GR = glutathione reductase;

GRO/KC = growth-related oncogene/keratinocyte-derived cytokine;

GSH = glutathione;

GST = glutathione S-transferase;

HDAC1 = histone deacetylase 1;

HSP70 = heat shock protein 70;

IκBα = inhibitory kappa B-alpha;

IKKα = IκB kinase-alpha;

IL = interleukin;

iNOS = inducible nitric oxide synthase;

JNK = c-Jun NH_2-terminal kinase;

LPS = lipopolysaccharide;

MMP = matrix metalloproteinase;

NF-κB = nuclear factor-kappaB;

NMBA = *N*-nitrosomethylbenzylamine;

Nrf2 = nuclear factor E2-related factor 2;

3-NT = 3-nitrotyrosine;

5′-NT = 5′-nucleotidase;

8-OHdG = 8-hydroxy-2'-deoxyguanosine;

O^6-meGua = O^6-methylguanine;

PB = phenobarbital;

PARP = poly(ADP-ribose) polymerase;

PCA = protocatechuic acid;

PCNA = proliferating cell nuclear antigen;

PGE_2 = prostaglandin E_2;

QR = quinone reductase;

ROS = reactive oxygen species;

SCE = squamous cell carcinoma of the esophagus;

TNF-α = tumor necrosis factor-alpha;

VEGF = vascular endothelial growth factor

ABSTRACT

Epidemiological reports as well as experimental studies have demonstrated the significant health benefits provided by regular berry consumption. Berries possess both

prophylactic and therapeutic potential against several chronic illnesses, such as cardiovascular, neurodegenerative and neoplastic diseases. Berries owe their health benefits to phytoconstituents, such as polyphenolic anthocyanins, ellagic acid and a diverse array of phytochemicals bestowed with potent antioxidant and anti-inflammatory effects as well as the ability to engage a multitude of signaling pathways. This review highlights the principal chemical constituents present in berries and their primary molecular targets. The article presents and critically analyzes the chemopreventive and therapeutic potential of berry extracts, fractions and bioactive components on various cancers of the gastrointestinal tract (GIT), including esophageal, stomach, intestinal and colorectal cancers as well as cancers of the upper aerodigestive tract, such as oral cancer. The current status of clinical studies evaluating berry products in several aforementioned cancers is presented. Various emerging issues including dose-ranging and dosage forms, the role of synergy and the usage of combination therapy as well as other relevant areas essential for the development of berry phytoconstituents as mainstream chemopreventive and therapeutic agents against aerodigestive and GIT cancers are critically discussed.

Keywords: Berry, aerodigestive and gastrointestinal tract, cancer, chemoprevention, treatment

INTRODUCTION

Gastrointestinal cancer is cancer of the organs of the digestive system, which include those of the esophagus, stomach, colon, rectum, and anus. Although small intestine represents a major component of the gastrointestinal tract (GIT), cancer occurs only rarely in this organ. Cancers of the GIT are among the most common cancers and are a leading cause of cancer death worldwide. In the United States, they comprise 17.5% of the estimated new cancer cases, with 290,200 new cases and 144,570 deaths anticipated in 2013 (Siegel et al., 2013). The associated risk factors, pattern of incidence, prevalence, and prognosis vary among the organ sites. Some of the risk factors can be abated, and in some cases the cancers are preventable with proper screening. A large number of GIT cancers are curable when detected and treated at an early stage and also the mortality rates can be reduced.

Oral cancer is defined as neoplasms involving the oral cavity, which begins at the lips and ends at the anterior pillar of the fauces. Cancers of the oral cavity and pharynx represent approximately 3% of all malignancies in men and 2% of all malignancies in women in the United States (Neville and Day, 2002). Worldwide**,** it is estimated to be the sixth most common cancer, prevalence being highest in India (Zakrzewska, 1999). Squamous cell carcinoma, which arises from the oral mucosal lining, is the most common intra-oral malignancy and accounts for over 90% of oral tumors. Tobacco in all forms, including smoking (e.g., cigarettes, cheroots, loose tobacco in pipes) and non-smoking (e.g., snuff, betel quid, toombak, shammah) is carcinogenic in the upper aerodigestive tract and a strong association between oral cancer and tobacco

consumption is well established (Johnson, 2001). In spite of the ready accessibility of the oral cavity to direct examination, mortality remains high, since most cases are detected in a late stage. The survival rate for oral cancer remains unchanged over the past few decades. While the prognosis for cancer of the lip is good, the prognosis for intra-oral squamous cell carcinoma remains poor (Shah and Gil, 2009).

Esophageal cancer accounts for about 2% of all malignant tumors worldwide and is a treatable, but rarely curable**,** cancer. It has two major histologic types, squamous cell carcinoma of the esophagus (SCE), arising from squamous epithelium that undergoes inflammatory, atrophic, and dysplastic changes and adenocarcinoma of the esophagus (ACE) arising through metaplastic intestinal-type changes that replace the squamous epithelium; both types have a poor prognosis (Brown et al., 2002). Esophageal cancer is relatively uncommon in the United States, with 17,990 new cases and 15,210 deaths anticipated in 2013 (Siegel et al., 2013). Smoking and ingestion of tobacco condensates is associated with an increased risk of both SCE and ACE, and directly correlates with the quantity of cigarettes smoked per day and the duration of smoking (Abraham et al., 2011). About 50% of all SCE worldwide occurs in high-risk provinces in China and is associated with the consumption of salty and moldy foods, hot beverages, *N*-nitrosamine carcinogens, and deficiencies in dietary minerals and vitamins (Stoner and Gupta, 2011). The major risk factor for ACE is gastro-esophageal reflux disease that is associated with obesity, hiatal hernia and the consumption of certain foods (Kresty et al., 2006). The incidence of ACE has increased dramatically in the Western world in the past two decades and is currently on the rise in other parts of the world that are

adopting the Western diet (Spechler, 2002). Finally, a history of radiotherapy to the mediastinum, such as the treatment of breast cancer and other neoplasms, predisposes patients to both histologic types of esophageal cancer (Enzinger and Mayer, 2003).

Recent estimates of global cancer incidence indicate that gastric cancer ranks second in cancer deaths across the world (Takahashi et al., 2013). The estimated incidence of gastric cancer in the United States in 2013 is 21,600 cases with 10,990 deaths (Siegel et al., 2013). Adenocarcinomas represent 90–95% of all gastric malignancies. Over the past 25 years, the number of gastric cancers occurring at the gastroesophageal junction and in the cardia has dramatically increased (Griffin-Sobel, 2007). Diffuse gastric cancer is associated with hereditary factors and does not appear to occur in the setting of intestinal metaplasia or dysplasia. Intestinal-type gastric cancer occurs in younger patients, is more frequently endemic, and is associated with inflammatory changes and with *Helicobacter pylori* infection (Alberts and Goldberg, 2009). Gastric cancer has been linked to the consumption of red meats, high-salt and high-carbohydrate diets, spices, smoked foods, and low consumption of vitamins A, C, and E. Pernicious anemia, achlorhydria, and atrophic gastritis, previous gastric resection, mucosal dysplasia and gastric polyps are other factors involved in the development of gastric cancer. Gastric cancers are most frequently discovered in advanced stages, and the prognosis of advanced gastric cancer remains poor, with curative surgery regarded as the only option for cure, followed by chemotherapy and radiotherapy.

Although the small intestine constitutes three-fourths of the length and approximately 90% of the surface area of the GIT, malignant tumors of the small intestine are rare. It is estimated that 8,810 new cases of small intestine cancer as well as 1,170 deaths may have occured in United States in 2013 (Siegel et al., 2013). The main histological subtypes include adenocarcinomas, carcinoid tumors, lymphoma and sarcoma. Very little is known about its etiology. The risk factors include other medical conditions, including Crohn's disease, celiac disease, familial adenomatous polyposis, Peutz-Jeghers syndrome, cholecystectomy, peptic ulcer disease, and cystic fibrosis (Neugut et al., 1998). Several behavioral risk factors, including consumption of red or smoked meat, saturated fat, obesity and cigarette smoking have also been suggested. The prognosis for cancer of the small intestine is poor with less than 30% relative survival at 5 years (Pan and Morrison, 2011).

Colorectal cancer (CRC) is a preventable, treatable, and often curable cancer, common in both men and women. About one million cases of CRC are diagnosed worldwide each year (Alberts and Goldberg, 2009). In the United States, it is the third most common type of cancer in men and women, with an estimated 73,680 new cases in men and 69,140 cases in women to occur in 2013 (Siegel et al., 2013). Multiple factors are associated with the transformation of healthy colorectal mucosa to cancer, including high-fat diet, inflammatory bowel diseases (ulcerative colitis and Crohn's disease), genetic factors (family history, inherited mutations in the adenomatous polyposis coli gene and the MLH1 and MSH2 DNA mismatch repair genes, and acquired genetic abnormalities like RAS gene point mutation, c-MYC gene

amplification) and smoking are factors involved in the development of CRC (Janout and Kollárová, 2001). Maintaining a low body mass index and exercising regularly correlate with lower incidence rates. Seventy percent of patients have localized disease, and surgery is the primary form of treatment and results in a cure in more than 50% of the patients. However, local or distant relapses after complete resection develop in many patients, and those with the highest risk of recurrence receive chemotherapy. Polyp removal and fecal occult blood testing reduced the incidence of CRC by 75% and 20%, respectively (Griffin-Sobel, 2007).

Anal cancers are uncommon with only 7,060 incident cases expected to arise in the United States in 2013. In North America, squamous cell carcinomas are the most common histology, constituting around 80% of anal cancers. Anal cancer appears to be more similar etiologically to genital malignancies than to malignancies of the gastrointestinal tract. Risk factors include a history of multiple sexual partners, female gender, human papillomavirus infection, cigarette smoking, genital warts, anal intercourse, and infection with the human immunodeficiency virus (Johnson et al., 2004). Treatment consists of surgery, radiation, and chemotherapy (Griffin-Sobel, 2007).

Dietary factors are among the most important environmental factors implicated in GIT cancers. It has been estimated that around 10-70% of all cancers are attributable to diet and that up to 90% of colorectal cancer may be avoidable through alterations in the diet and life style (Kim and Mason, 1996). Use of anti-inflammatory drugs, particularly aspirin, sulindac, and other non-steroidal anti-inflammatory drugs, is associated with a

decreased incidence of CRC, esophageal and gastric cancers. These drugs appear to mediate their protective effects through prevention of adenoma formation and by regressing adenomatous polyps, both sporadic polyps and heritable polyps, such as in patients with FAP (Griffin-Sobel, 2007; Hecht, 2006).

The increasing incidence rate and poor prognosis of patients with digestive tract cancers has focused interest in the management of these diseases through dietary chemoprevention which is the use of naturally occurring phytochemicals to reverse, suppress, or prevent the progression of premalignant lesions to malignant cancers. An impressive amount of data generated during the last several decades has shown digestive tract cancer preventive and therapeutic potential of a large number of dietary phytochemicals (Bishayee, 2009; Bishayee et al., 2012; Chung et al., 2013; Darvesh et al., 2012; Darvesh and Bishayee, 2013; Derry et al., 2013; González-Vallinas et al., 2013; Gullett et al., 2010; Madka and Rao, 2013). In this review, we have examined the available evidence to understand the full potential of berry fruits in the management of cancers of the digestive tract. Several prior publications (Brown et al., 2012; 2014; Folmer et al., 2014; Giampieri et al., 2014; Schreckinger et al., 2010; Seeram, 2008a; Stoner, 2009; Stoner et al., 2008a; Stoner and Seeram, 2011; Stoner and Wang, 2013) provide an excellent overview of broad area of research related to berries and all types of cancers. Nevertheless, there exists a need for a systematic, comprehensive, and critical evaluation of up-to-date literature on berry constituents in digestive tract cancer. Since berry phytochemicals can be directly absorbed in the oral cavity as well as the gastrointestinal tract following oral consumption, they may confer better cancer

preventive and antitumor efficacy in these organs compared to distant anatomical sites. Accordingly, this work was performed to collect, arrange, present, and critically analyze available *in vitro*, *in vivo* and clinical studies to understand the utility of berry fruits in the aerodigestive and gastrointestinal tract cancer prevention and therapy.

BERRY PHYTOCHEMICALS

According to botanical definition, the true “berry” is a simple fruit having seeds and edible pulp produced from a single ovary (e.g, cranberry, blueberry, gooseberry, grapes, tomato, avocado, and banana). In common use, berries are more broadly recognized as small, round or semi-oblong, usually brightly colored, sweet or sour, juicy edible fruit. Like other fruits, berries contain micro- and macro-nutrients such as vitamins, minerals, and fiber; however, their biological properties have been largely attributed to their high levels of various phenolic compounds (Schreckinger et al., 2010). Edible berries are rich sources of bioactive compounds, such as anthocyanins and flavonoid glycosides (Zafra-Stone et al., 2007), which are responsible for the red, violet, purple, and blue color of the fruits, and various phenolics and organic acids (Puupponen-Pimiä et al., 2005), which have antimicrobial activities against human pathogens. Several scientific studies have demonstrated that consumption of berries has beneficial effects on several chronic conditions, including obesity, cancer, cardiovascular, neurodegenerative diseases, and infectious diseases such as refractory urinary infections and infection with *H. pylori* (Alvarez-Suarez et al., 2014; Battino et al., 2009; Gopalan et al., 2012; Matsushima et al., 2008; Schreckinger et al., 2010; Seeram, 2008b).

Berries, such as blackberry (Rubus sp.), bilberry (*V. myrtillus*), strawberry (*Fragaria ananassa*), lingonberry (*V. vitis-idea*), gooseberry (*Ribes uva-crispa*), black currant (*Ribes nigrum*), blueberry (*V. corymbosum*), chokeberry (*Aronia melanocarpa*), cranberry (*V. macrocarpon*), bayberry (*Myrica sp.*), raspberry (*Rubus ideaus*), black raspberry (*Rubus occidentalis*) and cloudberry (*Rubus chamaemorus*), are usually consumed in fresh and in processed forms, such as juices, jams, and purées in the human diet. Some of the known chemopreventive agents present in berries include vitamins A, C, E and folic acid; calcium and selenium; β-carotene, α-carotene, and lutein; phytosterols, such as β-sitosterol and stigmasterol; triterpene esters; and phenolic compounds (Seeram, 2008a; Stoner, 2009). Phenolic compounds including flavonoids (anthocyanins, flavonols, flavanols and isoflavonoids), tannins (proanthocyanidins, ellagitannins and gallotannins), stilbenoids, phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and lignans are largely implicated in the bioactivities of these fruits and have thus received considerable interest. The structures of the predominant berry phenolic compounds are summarized in Fig. 1.

Phenolic compounds are secondary metabolites ubiquitous in all higher plants, with fruits, including berries, being the major source in our diets. All phenolic phytochemicals are derived from a common biosynthetic pathway, incorporating precursors from both the shikimate and/or the acetate-malonate pathways. Phenolic compounds present in berries have numerous defense functions in those plants, including protection of the plants from microbial and viral infections. Factors such as species, variety, geographic region, storage conditions, ripeness, and climate, may affect the concentration of

phenolics in berries. In general, the phenolic content and antioxidant capacity of wild berries are higher compared with domesticated and genetically derived berries (Burns Kraft et al., 2008). Red currant, lingonberry, blueberry, bilberry and blackberry have remarkably high contents of phenolics per gram fresh weight (Paredes-López et al., 2010).

Anthocyanins, the flavonoid phytopigments localized in the exocarp layer of the fruit's skin, are responsible for the bright red, blue and purple colors of berries and other plants. The principal anthocyanin found in most red berries is cyanidin-3-*O*-glucoside. Pelargonidin, peonidin and delphinidin glucosides are the other major anthocyanin compounds of most edible berries (Wu et al., 2006; Zafra-Stone et al., 2007). Blueberry, blackberry, bilberry and cranberry have high amounts of total anthocyanin compounds per gram fresh weight (Paredes-López et al., 2010). The major anthocyanins in cranberry are galactosides and arabinosides of cyanidin and peonidin along with low concentrations of cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, delphinidin-3-*O*-galactoside, delphinidin-3-*O*-arabinoside (Côté, et al., 2010). The major anthocyanins in raspberries and blackberries are cyanidin derivatives, while pelargonidin-3-*O*-glucoside is predominant in strawberries. The major components in blueberries are malvidin-3-*O*-arabinoside and the 3-*O*-galactosides of cyanidin, delphinidin, petunidin and malvidin (Del Rio et al., 2010).

Myricetin, quercetin and kaempferol are the major flavonols of edible berries. Cranberry, lingonberry and black currant have the highest flavonol contents (~50–200 mg/kg fresh weight). Quercetin and kaempferol are abundant in berries, such as

bilberry, cranberry, and chokeberry. Quercetin was found to be highest in bog whortleberry (158 mg/kg) and bilberry (17–30 mg/kg). In black currant cultivars, myricetin was the most abundant flavonol (89–203 mg/kg), followed by quercetin (70–122 mg/kg) and kaempferol (9–23 mg/kg) (Häkkinen et al., 1999). The flavonol glycosides present in berries include quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside and quercetin-3-*O*-xylosylglucuronide, myricetin-3-*O*-glucoside, myricetin-3-*O*-galactoside and myricetin-3-*O*-rutinoside (Del Rio et al., 2010). Berries also contain the flavan-3-ol monomers (+)-catechin and (−)-epicatechin as well as dimers, trimers and polymeric proanthocyanidins, cranberries being a rich source of these compounds.

Caffeic and ferulic acids, hydroxylated derivatives of cinnamic acid, are the most common phenolic acids found in berries. They are mostly esterified with other molecules as carbohydrates and organic acids (Mattila et al., 2006; Paredes-López et al., 2010). The other simple phenolics in berries include chlorogenic, sinapic, and p-coumaric acids along with hydroxylated derivatives of benzoic acid, such as gallic and vanillic acids (Vattem et al., 2005). Hydroxycinnamoylglucosides are found in most berries, but usually in low concentrations. Chokeberry contains high amounts of hydroxycinnamic derivatives, such as chlorogenic acid, which is an ester between caffeic acid and quinic acid (Shahidi and Neczk, 1995). Cloudberries, raspberries and strawberries, all belonging to the family Rosaceae, have relatively high content of hydroxybenzoic acids compared to that of berries from other families (Kähkönen et al., 2001). Galloylglucose is present in blackberries and black currants (2-7 ppm) while 5'-

galloylquinic acid (theogallin) is found in strawberries, raspberries, red and black currants (Schuster and Herrmann, 1985).

Phenolic compounds, such as resveratrol, pterostilbene, piceatannol and other naturally occurring stilbenes/stilbenoids, are known to be strong antioxidants with cancer chemopreventive activities (Niesen et al., 2013). Resveratrol was found in lowbush blueberry (*V. angustifolium*), sparkleberry (*V. arboretum*), rabbiteye blueberry (*V. ashei*), highbush blueberry (*V. corymbosum*), Elliott's blueberry (*V. elliottii*), cranberry, bilberry, deerberry (*V. stamineum*), lingonberry, and partridgeberry (*V. vitis-ideae* var. minor) at levels between 7 and 5,884 ng/g dry sample. Pterostilbene was found in rabbiteye blueberry and in deerberry at levels of 99−520 ng/g dry sample. Piceatannol was found in highbush blueberry and deerberry at levels of 138−422 ng/g dry sample (Rimando et al., 2004).

Flavonoids and phenolic acids form the building blocks for polymeric tannins, which are classified into hydrolyzable and condensed tannins. Hydrolyzable tannins are either gallotannins or ellagitannins. Ellagitannins are esters of glucose with hexahydroxydiphenic acid and when hydrolyzed they yield ellagic acid, the active chemopreventive component in the ellagitannins (Stoner and Mukhtar, 1995). Most of the ellagic acid in berries is in present in the ellagitannins although some is in free form. Red raspberry, arctic bramble and cloudberry are rich in ellagitannins (Häkkinen et al., 2000). Hydrolysis of the ellagitannins in lyophilized berries revealed high concentrations of ellagic acid in red raspberries (15 - 58 mg/kg), strawberries (6.3 -18 mg/kg) and blackberries (15 - 88 mg/kg) (Amakura et al., 2000; Daniel et al., 1989) and in black

raspberries (16.6 – 22.5 mg/kg) (Stoner, 2009). Indeed, ellagic acid constitutes >50% of total phenolics in strawberries and raspberries. Berries also contain condensed tannins (proanthocyanidins), polymers of flavan-3-ols and flavan-3,4-diols or their mixtures, which provide the characteristic bitter taste to many berries (Puupponen-Pimiä et al., 2005).

Berries, such as lingonberry, strawberry and cranberry, are also rich in diphenolic compounds called lignans (Mazur et al., 2000). The lignans are concentrated in the seeds of the berries. Cloudberry seeds are a lignan-rich food source followed by blackberries and cranberries, and most of the berry species are more lignan-rich than the cereal brans. Medioresinol, syringaresinol, lariciresinol, pinoresinol and secoisolariciresinol are the most abundant lignans in berries (Smeds et al., 2012).

PRECLINICAL STUDIES ON DIGESTIVE TRACK CANCER PREVENTIVE AND ANTICANCER EFFECTS OF BERRIES

Although a wide variety of berries are consumed worldwide, we have primarily focused our attention on studies carried out on aerodigestive and gastrointestinal track cancer chemopreventive and therapeutic effects of berries commercially cultivated and commonly consumed in North America, such as blackberries, blueberries, black and red raspberries, cranberries and strawberries. We have also presented limited number of studies on “niche-cultivated” and forest/wild berries, including, bilberries, black and white currants, arctic bramble berries, chokeberries, cloudberries, elderberries,

gooseberries, lingon berries, açaí berries and wolfberries, which are also consumed in other parts of the world. Studies on grapes, cherries (which may not be considered as berries), and “berry-type” fruits, such as pomegranate, are considered out of the scope for this review. The interested readers are directed to excellent reports on health benefits of consumption of grapes and grape products (Yang and Xiao, 2013; Effie and Antonia, 2014) as well as studies that documented chemopreventive and anticancer effects of grapes against gastrointestinal track cancers (Dinicola et al., 2013; Kountouri et al., 2013; Cheah et al., 2014).

In vitro studies

Oral cancer

Seeram and coinvestigators (2004) evaluated the antiproliferative activity of total cranberry extract versus its individual fractions using KB and CAL27 human oral cancer cell lines (Table 1). The total polyphenol fraction was found to be the most active fraction against both cell lines with 96 and 95% growth inhibition of KB and CAL27 cells, respectively. The same research laboratory also reported the growth inhibitory effects of several berry extracts against the aforementioned tumor cells *in vitro* (Seeram et al., 2006). Black raspberry semipurified fractions as well as isolated components, such as ferulic acid and β-sitosterol, inhibited the growth of oral premalignant and malignant cells by targeting cell cycle regulatory proteins (Han et al., 2005). Another study employed cell lines isolated from human oral squamous cell carcinoma to investigate

the effects of a freeze-dried black raspberry ethanol extract on tumor cell growth characteristics. The results demonstrate that the extract suppressed proliferation without perturbing viability, inhibited translation of the angiogenic cytokine vascular endothelial growth factor (VEGF), decreased iNOS activity and induced apoptosis and terminal differentiation (Rodrigo et al., 2006). Strawberry crude extracts and purified phenolic compounds were evaluated for antioxidant and antiproliferative activities using KB and CAL27 human oral cancer cells. Crude extracts and pure compounds, such as cyanidin-3-*O*-glucoside, pelargonidin, pelargonidin-3-*O*-rutinoside, kaempferol, quercetin and ellagic acid, inhibited the growth of both cell lines and this was associated with an antioxidant mechanism of action (Zhang et al., 2008).

Esophageal cancer

A cranberry proanthocyanidin-rich extract significantly inhibited the viability and proliferation of SEG-1 human esophageal adenocarcinoma cells in time- and concentration-dependent manner. The extract also induced cell cycle arrest at the G_1 checkpoint, reduced the percentage of cells in S-phase and triggered apoptotic cell death (Kresty et al., 2008). An ethanolic extract of black raspberry and two active components, namely cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside, selectively caused significant growth inhibition and induction of apoptosis in a highly tumorigenic rat esophageal cell line (RE-149DHD), but not in a weakly tumorigenic line (RE-149). While the ethanolic extract did not alter COX-2 and iNOS expression in RE-148DHD

cells, both anthocyanins downregulated the expressions of these genes (Zikri et al., 2009).

Stomach cancer

Black currant, raspberry, cranberry and gooseberry juice displayed substantial antiproliferative activities against AGS human gastric adenocarcinoma cells (Boivin et al., 2007). Similarly, an aqueous extract from red raspberry exhibited killing of AGS cells (God et al., 2010). Malvidin, a berry anthocyanidin, exhibited potent antiproliferative effect on AGS cells accompanied by cell cycle arrest at the G_0/G_1 phase, apoptosis induction, caspase-3 activation, poly(ADP-ribose) polymerase (PARP) proteolysis and elevation of Bax/Bcl-2 ratio. Additionally, malvidin treatment significantly increased the p38 kinase expression and inhibited extracellular signal-regulated kinase (ERK) activity in AGS cells (Shih et al., 2005). A subsequent study confirmed the antiproliferative effect of malvidin against AGS cells and found a similar result with another berry anthocyanidin, pelargonidin (Zhang et al., 2005).

Liver cancer

Extracts from eight strawberry cultivars containing varying amounts of flavonoids and anthocyanins exhibited significant antiproliferative effects against HepG2 human liver cancer cells in a dose-dependent manner. Interestingly, no relationship was found between the observed antiproliferative activity and antioxidant content (Meyers et al., 2003). The phenolic acid and anthocyanin fractions from blueberry showed inhibition of HepG2 cell population growth with induction of apoptosis (Yi et al., 2006). Bishayee and

colleagues (2010) investigated the antiproliferative effect of black currant skin extract, containing cyanidin-3-*O*-rutinoside, against HepG2 cells. The extract registered a potent cytotoxic effect and the result was more pronounced than that of delphinidin and cyanidin. Another laboratory reported antiproliferative activities of isolated cranberry phytochemicals, namely quercetin, ursolic acid and 3,5,7,3′,4′-penahydroxyflavonol-3-*O*-β-D-glucopyranoside, against HepG2 cells. The first two compounds also exhibited antioxidative activities (He et al., 2006). Delphinidin, cyanidin and malvidin exhibited strong growth inhibitory effects against HepG2 human hepatoma cells, but were less effective against Hep3B cells. Delphinidin induced apoptotic cell death through regulation of Bcl-2 family molecules and activation of c-Jun NH_2-terminal kinase (JNK) cascade (Yeh and Yen, 2005). In an interesting study, another group of researchers found that delphinidin, extracted and purified from bilberries, caused growth retardation in hepatocellular carcinoma cells through macroautophagy, but not apoptosis (Feng et al., 2010).

Colon cancer

Among ethanol extracts of 10 edible berries, bilberry extract was found to be the most effective in inhibiting the growth of HCT116 human colon carcinoma cells (Katsube et al., 2003). An anthocyanin rich bilberry extract was found to reduce the viability HT-29 colon cancer cells possibly via amelioration of oxidative DNA damage, suppression of ROS level and elevation of GSH content (Schantz et al., 2010). Commercially prepared bilberry and chokeberry anthocyanin-rich extracts were investigated for their

potential antineoplastic activity against colon cancer cells. Both extracts inhibited the growth of HT-29 cells with chokeberry extract being the most potent inhibitor (Jing et al., 2008; Zhao et al., 2004). The antiproliferative effect of anthocyanin-rich chokeberry extract was associated with cell cycle blockage at G_1/G_0 and G_2/M phases, increased expression of $p21^{WAF1}$ and $p27^{KIP1}$ and decreased expression of cyclin A and cyclin B. A 35% decrease in the COX-2 gene expression was observed in HT-29 cells treated with the extract for 24 h (Malik et al., 2003). Using standard chromatographic techniques, a warm-water extract of cranberry presscake (the material remaining following squeezing juice from the berries) demonstrated antiproliferative activity against HT-29 cells (Ferguson et al., 2004). The antiproliferative effects of total cranberry extract and various fractions were evaluated against a panel of colon cancer cell lines, including HT-29, HCT-116, SW480 and SW620. The total polyphenol fraction displayed superior antitumor efficacy against all cell lines to any other cranberry product tested (Seeram et al., 2004). Similar cytotoxic effects against colon cancer cells were observed with other berry extracts (Seeram et al., 2006). Proliferation of HT-29 cells was also inhibited by an extract from black chokeberry, lingonberry or raspberry and these effects were correlated with the levels of carotenoids and vitamin C (Olsson et al., 2004). Crude extracts of blueberries and different fractions inhibited the proliferation of two human colon cancer cell lines, namely HT-29 and Caco-2. The anthocyanin fraction also resulted in substantial increase in DNA fragmentation, indicative of apoptosis (Yi et al., 2005). Anthocyanin-enriched fractions from blueberries induced apoptosis in HT-29

cells with a concurrent increase in caspase-3 activity and decrease in QR and glutathione S-transferase (GST) activities (Srivastava et al., 2007).

Wu and colleagues (2007) compared the antiproliferative potential of several berry extracts containing different profiles of phenolic compounds, such as anthocyanins, flavonols and ellagic acid, in human colon cancer HT-29 cells. The degree of cell growth inhibition was as follows: bilberry > black currant > cloudberry > lingonberry > raspberry > strawberry. An anthocyanin-rich extract (mainly containing delphinidin and malvidin) prepared from *V. uliginosum* L., a type of blueberry found in Chinese Changbai Mountains, dose-dependently repressed the proliferation of COLO205 human colorectal cancer cells via induction of apoptosis (Zu et al., 2010). The effects of extracts from five cultivars of strawberries on the proliferation of HT-29 colon cancer cells were investigated. The extracts from organically grown strawberries had a higher antiproliferative activity at the highest concentration than the conventionally grown types, which could be related to the presence of higher content of secondary metabolites with anticarcinogenic properties in the organically grown fruits (Olsson et al., 2006). In another study, extracts prepared from strawberries treated with essential oils, namely thymol, menthol or eugenol, exhibited stronger antiproliferative effects against HT-29 cells than those from untreated fruits (Wang et al., 2007). Strawberry crude extract and phenolic fractions inhibited the growth of HT29 and HCT-116 colon cancer cells possibly due to potent antioxidative mechanism (Zhang et al., 2008). McDougall and colleagues (2008) confirmed the antiproliferative effects of polyphenol-

rich strawberry extract in a different colon cancer cell line (CaCo-2) and showed similar results with arctic bramble, cloudberry and lingonberry.

A “colon-available” raspberry extract was prepared that contained phytochemicals surviving digestion that resembled the physiochemical conditions of the upper gastrointestinal tract. Although this polyphenol-rich extract had no significant effect on cytotoxicity against HT-29 cells, it caused noteworthy protective effects against DNA damage induced by hydrogen peroxide and decreased the population of cells in G1 phase of the cell cycle. Additionally, the extract significantly suppressed the invasion of HT-115 colon cancer cells (Coates et al., 2007). Three days of treatment with black raspberry-derived anthocyanins at 0.5, 5 and 25 μg/ml abrogated the proliferation of and induced apoptosis in various colon cancer cell lines. The anthocyanins demethylated tumor suppressor genes, such as CDKN2A, SFRP2, SFRP5 and WIF1, through inhibition of DNA methyl transferase 1 (DNMT1) and DNMT3B (Wang et al., 2013a). Red raspberry extract was found to exert antineoplastic effect in LoVo colon cancer cells associated with weak antioxidant effect and lack of apoptosis (God et al., 2010).

Black currant juice impeded the growth of CaCo-2 cells though the mechanism of such action was not studied (Biovin et al., 2007). Another study reported that extracts from black currant press residue containing anthocyanins and polyphenols retarded the proliferation of several colon cancer cells, namely Caco-2, HT-29 and HCT-116. In HT-29 cells, the antiproliferative effect was associated with induction of apoptosis (Holtung et al., 2011).

Cyanidin, but not its glycosides, was found to be a potent inhibitor of mitogen-induced metabolic activity, intracellular CA^{2+} accumulation and cellular growth of HT-29 human colon carcinoma cells (Briviba et al., 2001). In another study, cyanidin exhibited antiproliferative effect only in normal fibroblasts, whereas delphinidin affected the proliferative capacity of normal as well as CaCo-2 colon carcinoma cells. Delphinidin also showed cell cycle-regulatory and proapoptotic activities in tumor cells [98]. Using HCT-116 human colon cancer cells, Yun et al. (2009) showed that delphinidin inhibited tumor cell growth by causing G_2/M cell cycle arrest, apoptosis induction and suppression of the activated NF-κB pathway. Cvorovic and colleagues (2010) found that cyanidin and delphinidin were cytotoxic in the metastatic human colorectal cell lines, namely LoVo and LoVo/ADR, in which both compounds accumulated cellular ROS, inhibited glutathione reductase (GR) and depleted glutathione (GSH). Another study found tumor cell growth-inhibitory effects of malvidin and pelargonidin against HCT-116 cells (Zhang et al., 2005). Five anthocyanidins and eleven catechins were evaluated for their potential antitumor effects against HCT-116 colon cancer cells. At 100 μM concentrations, most of these compounds were ineffective in inhibiting tumor cell proliferation. In contrast, at 50 μM concentration, (−)-gallocatechin and (−)-gallocatechin gallate exhibited substantial inhibition of colon cancer cell growth (Seeram et al., 2003). In Caco-2 colon cancer cells, ellagic acid inhibited cell proliferation and fetal bovine albumin-induced cell migration. Mechanistic studies showed apoptosis induction with concomitant decrease in the levels of pro-matrix metalloproteinase-2 (pro-MMP-2), pro-matrix metalloproteinase-9 (pro-MMP-9) and VEGF (Losso et al., 2004). In a separate

study, ellagic acid provoked antiproliferative effect in Caco-2 cells which was accompanied by downregulation of cyclins A and B, upregulation of cyclin E, cell cycle arrest in S phase and induction of apoptosis via the intrinsic pathway through activation of initiator caspase-9 and effector caspase-3 (Larrosa et al., 2006).

In vivo studies

Oral cancer

The potential chemopreventive effect of black raspberry was investigated using 7,12-dimethylbenz(a)anthracene (DMBA)-induced hamster cheek pouch tumors (Table 2). Dietary administration of freeze-dried black raspberries to hamsters at a concentration of 5% of the diet inhibited the incidence, total number, multiplicity and size of tumors, possibly by reducing the formation of the procarcinogenic adduct formed by DMBA (Casto et al., 2005).

Esophageal cancer

A freeze-dried strawberry preparation was evaluated for its ability to inhibit NMBA-induced esophageal tumorigenesis in rats when added to the diet at concentrations of 5 and 10%. Although neither concentration of strawberries reduced the tumor (papilloma) incidence, both concentrations lowered tumor multiplicity with parallel reductions in the formation of O^6-methylguanine (O^6-meGua) in esophageal DNA (Stoner et al., 1999). Similar effects were found when rats were fed with the strawberry diets before and after

NMBA dosing or only after the carcinogenic challenge. Interestingly, the 10% strawberry diet was not more effective in reducing esophageal papilloma development than the 5% berry diet when the diets were administered either before or after NMBA dosing (Carlton et al., 2001).

A similar food-based chemopreventive approach was taken to evaluate the inhibitory potential of freeze-dried black raspberries against NMBA-induced esophageal tumorigenesis in rats during the initiation and post-initiation phases of carcinogenesis. Feeding 5 and 10% black raspberries via food 2 weeks prior to NMBA treatment and throughout 30 weeks significantly reduced tumor multiplicity. Administration of raspberry diet after NMBA treatment inhibited tumor progression as evidenced by significant reductions in the formation of preneoplastic esophageal lesions, decreased tumor incidence and multiplicity and suppressed cellular proliferation based on proliferating cell nuclear antigen (PCNA) labeling index. The 5% black raspberry diet was found to be just as effective as the 10% black raspberry diet in reducing tumor development in the rat esophagus (Kresty et al., 2001). Subsequent studies from the same laboratory found that freeze-dried black raspberries, at 5% of the diet, inhibited NMBA tumorigenesis by reducing mRNA and protein levels of COX-2, iNOS and c-Jun as well as the level of prostaglandin E_2 (PGE_2) in preneoplastic lesions (Chen et al., 2006a). An ancillary study revealed antiangiogenic mechanisms of black raspberry as evidenced from immunohistochemical analysis of esophageal microvessel density and reduced expression of VEGF in rats subjected to NMBA carcinogenesis (Chen et al., 2006b). Freeze-dried raspberries were also evaluated for their potential therapeutic effects

against NBMA-induced rat esophageal carcinogenesis. The results indicated that the berries had no effect on the development of NMBA-induced tumors in the rat esophagus or on animal survival when administered for 7 weeks beginning at the papilloma stage of tumor development (Stoner and Aziz, 2007). The esophagi of rats treated with NMBA exhibited significant hyperplasia and low-grade dysplasia in concert with marked infiltration of inflammatory cells. Diet supplemented with 5% freeze-dried black raspberry 2 weeks before and during the week of NMBA treatment protected the animals from the early stage of esophageal carcinogenesis. The investigators also found that 462 of the 2,261 NMBA-dysregulated genes in rat esophagus were restored to near-normal levels of expression by black raspberry. Moreover, the berry product positively modulated 53 common genes, including genes involved in phase I and II xenobiotic metabolism, oxidative damage and oncogenes and tumor-suppressor genes that regulate apoptosis, cell cycle progression and angiogenesis (Stoner et al., 2008).

Diets containing either 5% whole black raspberries or an anthocyanin-rich fraction having the same amount of anthocyanins as were found in the 5% black raspberry diet were almost equally effective in reducing NMBA tumorigenesis in rats. This result indicated that the anthocyanins may account for a significant portion of the chemopreventive efficacy of black raspberries. All black raspberry products inhibited carcinogen-mediated cell proliferation, inflammation and angiogenesis, suggesting similar mechanisms of action by the different berry components (Wang et al., 2009). A subsequent study from the same research group showed that treatment with 5% black raspberry reduced the number of dysplastic lesions and the number and size of

esophageal papillomas during postinitiation phase of NMBA-induced esophageal tumorigenesis in rats. In both preneoplastic esophagus and papillomas, black raspberry modulated the mRNA expressions of genes associated with carbohydrate and lipid metabolism, cell proliferation, apoptosis and inflammation. Additionally, black raspberry modulated the expression of proteins involved in proliferation, apoptosis, inflammation, angiogenesis, invasion, adhesion and metastasis (Wang et al., 2011a).

Freeze-dried black raspberry, blackberry and strawberry administered in the diet before, during and after NMBA treatment inhibited rat esophageal carcinogenesis as evidenced by decrease**s** in tumor multiplicity, reduction in adduct formation, reduced proliferative indices, inhibition of preneoplastic lesion formation and downregulation of COX-2 and iNOS (Stoner et al., 2006). A post-initiation protocol was used to assess the relative ability of six different berry types to influence the progression stage of rat esophageal tumorigenesis. A diet containing 5% of either black or red raspberry, blueberry, strawberry, açaí or wolfberry was used in this experiment. All berry types were about equally effective in inhibiting NMBA-initiated tumorigenesis in the rat esophagus and simultaneously reduced the levels of serum cytokines, such as IL-5 and growth-related oncogene/keratinocyte-derived cytokine (GRO/KC), and increased serum antioxidant capacity (Stoner et al., 2010).

Mandal and Stoner (1990) reported the ability of ellagic acid to inhibit NBMA tumorigenesis in the rat esophagus. When administered in a diet at a concentration of 0.4 or 4 g/kg, ellagic acid significantly decreased the average number of NBMA-induced esophageal tumors. A follow-up study confirmed the chemopreventive efficacy of ellagic

acid and showed that ellagic acid in combination with 13-*cis*-retinoic acid was not effective in preventing NBMA-induced esophageal carcinogenesis (Daniel and Stoner, 1991). Ellagic acid when administered continuously before and after NMBA treatment or only after NMBA exposure exhibited inhibition of esophageal carcinogenesis in rats (Siglin et al., 1995; Stoner and Morse, 1997).

Liver cancer

Bishayee and coinvestigators (2011) reported chemopreventive efficacy of anthocyanin-rich black currant fruit skin extract against a classical two-stage rat liver carcinogenesis model. Initiation of hepatocarcinogenesis was performed by intraperitoneal injection of diethylnitrosamine (DENA) followed by promotion with phenobarbital (PB) in drinking water. Dietary administration of black currant extract 4 weeks prior to the initiation and continued for 22 consecutive weeks dose-dependently decreased the incidence, total number, multiplicity, size and volume of preneoplastic hepatic nodules, precursors of hepatocellular carcinoma. Mechanistic study revealed that black current extract suppressed abnormal hepatic proliferation and induced apoptosis via upregulation of Bax and downregulation of Bcl-2 expression. Under the same experimental conditions, black currant extract also abrogated the number and area of DENA-induced gamma-glutamyl transpeptidase-positive hepatic foci possibly through reversal of the overexpression of COX-2, heat shock protein 70 (HSP70) and HSP90 as well as blockade of nuclear translocation of NF-κB (Bishayee et al., 2013). The same laboratory also provided substantial evidence that black currant bioactive

phytoconstituents exert chemopreventive effect against DENA-induced hepatocarcinogenesis by induction of hepatic antioxidant and phase 2 xenobiotic-metabolizing enzymes through activation of nuclear factor E2-related factor 2 (Nrf2) signaling pathway (Thoppil et al., 2012).

Intestinal cancer

Mirtoselect, an anthocyanin mixture from bilberry and isolated cyanidin-3-*O*-glucoside were tested for their chemopreventive abilities against intestinal adenoma formation in the adenomatous polyposis coli (Min) ($Apc^{Min)}$ mouse, a genetic model of human FAP. Ingestion of either Mirtoselect or cyanidin-3-*O*-glucoside reduced adenoma load dose-dependently in the experimental animals. Accompanying pharmacokinetic study revealed presence of anthocyanins in the plasma, intestinal mucosa and urine (Cooke et al., 2006). Another *in vivo* study in F344 rats demonstrated that anthocyanin-rich extracts from bilberry and chokeberry significantly inhibited aberrant crypt foci (ACF) formation induced by azoxymethane (AOM). Cellular proliferation was suppressed by both berry extracts, whereas COX-2 mRNA expression was downregulated by the bilberry product (Lala et al., 2006). Dietary administration of three wild berries, namely bilberry, lingonberry and cloudberry, significantly reduced the total number of intestinal adenomas in Min/+ mice. Cloudberry resulted in decreased levels of nuclear β-catenin and cyclin D1 and lingonberry lowered the level of cyclin D1 in the large adenomas. Affymetrix microarrays revealed alterations in genes implicated in colon carcinogenesis, including the decreased expression of adenosine deaminase

(Ada), ecto-5′-nucleotidase (5′-NT) and PGE_2 receptor subtype EP4 (Mutanen et al., 2008).

Blueberry, blackberry and cranberry products exhibited chemopreventive effects against chemically-induced colon carcinogenesis in rats. Dietary feeding of 5% blueberry and blackberry and 20% cranberry juice instead of drinking water contributed to significant reductions in the formation of AOM-induced ACF in rats. Additionally, hepatic GST activities in rats exposed to blueberry and cranberry were significantly higher compared to control animals (Boateng et al., 2007). In a separate study, Min mice were fed a basal diet or a diet containing 10% freeze-dried white currant for 10 weeks. The white currant diet reduced the number of adenomas in the total small intestine and number and size of adenomas in the distal part of the small intestine. In contrast, white current increased the number of adenomas in the colon. White currant diet also reduced nuclear β-catenin and NF-κB protein levels in the adenomas in the distal small intestine (Rajakangas et al., 2008).

Freeze-dried black raspberries, at 5 and 10% of the diet, decreased the multiplicity of AOM-induced ACF, total tumors, tumor volumes and adenocarcinomas in rats, in part by modulating oxidative stress (Harris et al., 2001). Ulcerative colitis, a chronic inflammatory disease of the colonic mucosa, can dramatically increase the risk of colon cancer. The effect of a dietary intervention of freeze-dried black raspberry on disease severity in an experimental rodent model of ulcerative colitis using 3% dextran sodium sulfate (DDS) was evaluated. Dietary black raspberry markedly reduced DDS-induced acute injury to the colonic epithelium and colonic ulceration through anti-inflammatory

mechanisms as evidenced by suppressed tissue levels of COX-2 as well as proinflammatory cytokines TNF-α and IL-1β (Montrose et al., 2011). The same investigators also confirmed that dietary black raspberry reduced DDS-induced ulceration in mice by decreasing NF-κB p65 protein expression, leading to decreased DNMTs and thereby attenuating promoter methylation of tumor suppressor genes in the Wnt pathway (Wang et al., 2013b).

Using interleukin-10 knockout mice (a genetic model of ulcerative colitis), Wang and colleagues (2013c) showed that 5% black raspberry significantly reduced colonic ulceration with simultaneous suppression of nuclear translocation of β-catenin. Mechanistically, black raspberry modulated Wnt/β-catenin signaling pathway by sustaining correct methylation patterns and expression of Wnt pathway negative-regulatory genes.

To determine whether freeze-dried black raspberry could be used for the prevention of colorectal cancer, Bi and coresearchers (2010) administered a Western-style diet containing 10% black raspberry to two mouse models of human colorectal cancer, namely *Apc*1638+/- and *Muc*2-/-. A 12-week feeding of black raspberry significantly inhibited intestinal tumor formation in both models. Mechanistic studies revealed that black raspberry suppressed β-catenin signaling in *Apc*1638+/- and chronic inflammation in *Muc*2-/- mice. Cell proliferation was inhibited in both models.

CLINICAL STUDIES ON BERRIES AND CANCER

Several clinical trials have been conducted to evaluate chemopreventive and anticancer potential of various berry formulations in patients with cancers of oral cavity, esophagus and colon or with high risk of developing digestive tract cancer.

Mallery and colleagues (2007) developed a bioadhesive berry gel for application to the human oral mucosa and found that the gel composition was well-suited for absorption and penetration into the target oral mucosal tissue site. Utilizing this preparation, Shumway and coinvestigators (2008) conducted a clinical study to assess the effects of topical application of 10% freeze-dried black raspberry gel on oral intraepithelial neoplasia. The results showed histologic regression in a subset of patients as well as statistically significant reduction in loss of heterozygosity at tumor suppressor gene-associated loci. Additionally, none of the 27 patients developed berry-gel associated toxicities. The same group of investigators reported that the berry gel application uniformly suppressed genes associated with RNA processing, growth factor recycling and inhibition of apoptosis and significantly suppressed epithelial COX-2 levels. Moreover, in a subset of patients, the gel application induced genes associated with apoptosis and keratinocyte terminal differential and abrogated angiogenesis as evidenced from reduced microvessel density (Mallery et al., 2008).

Barrett's esophagus represents a preneoplastic condition in which the normal stratified squamous epithelium lining the esophagus is replaced by metaplastic columner epithelium containing goblet cells (Spechler, 2002). Barrett's esophagus confers a 30- to 40-fold increase in the risk of developing esophageal adenocarcinoma. A clinical study was conducted to investigate whether or not oral administration of black

raspberry powder (32 g for females and 45 g for males, once daily) would influence the progression of Barrett's lesions. The oral consumption of 45 g/day of black raspberry powder by humans is nearly equivalent to the daily consumption of a 5% black raspberry diet by rodents. Based on preliminary results, black raspberry consumption did not result in a reduction in the segment length of Barrett's lesions following 26 weeks of the study. Nevertheless, this study provides support that daily consumption of black raspberry promotes reductions in the urinary excretion of two markers of oxidative stress, namely 8-epi-prostaglandin F2α and 8-OHdG, in patients with Barrett's esophagus (Kresty et al., 2006). Dysplasia is a histological precursor of esophageal squamous cell carcinoma (Shimizu et al., 2009). Chen et al. (2012) conducted a randomized (noncomparative) phase II trial to evaluate the effect of freeze-dried strawberries in patients with esophageal dysplastic lesions in a high-risk area for esophageal cancer in China. Dietary strawberries (60 g/day for 6 months) significantly decreased the histological grade of precancerous esophageal lesions in more than 80% of patients. The strawberry powder was well tolerated with no toxic effects or serious adverse events. Ancillary mechanistic study revealed that the administration of strawberry powder significantly inhibited cell proliferation (Ki-67 labeling index) and reduced protein expression levels of iNOS, COX-2, pNF-κB p65 and pS6 (reminiscent of mammalian target of rapamycin activity).

Thomasset and colleagues (2009) investigated whether mirtocyan, an anthocyanin-rich standardized bilberry extract, causes pharmacodynamics changes consistent with chemopreventive efficacy and generates measurable levels of anthocyanins in blood,

urine and target tissue. Twenty-five colorectal cancer patients scheduled to undergo resection of primary tumor or liver metastases received mirtocyan at various amounts (1.4-5.6 g) daily for 7 days before surgery. Mirtocyan anthocyanins as well as methyl and glucuronide metabolites were detected in plasma, colorectal tissue and urine, but not in liver. In tumor tissue from all patients administered with mirtocyan, proliferation was suppressed compared with preintervention values. The low doses registered a small but nonsignificant reduction in circulating insulin growth factor I concentrations. Another phase I pilot study evaluated the effects of black raspberries on biomarkers of tumor development in the human colon and rectum. Biopsies of adjacent normal tissues and colorectal adenocarcinomas were taken from 20 patients before and after oral consumption of black raspberry powder (60 g/d) for 1-9 weeks. The methylation of three Wnt inhibitors, such as *SFRP2*, *SFRP5* and *W1F1*, upstream genes in Wnt pathway and PAX6a, a developmental regulatory gene, was modulated in a protective fashion by black raspberry in normal as well as colorectal tumoral tissues only in patients who received the berry treatment for an average of 4 weeks. Black raspberry also modulated the expression of genes associated with Wnt signaling, proliferation, apoptosis, and angiogenesis in a protective direction (Wang et al., 2011b). Plasma and biopsy samples of colorectal adenocarcinoma and adjacent normal-appearing tissues were collected before and during berry treatment from 24 patients who drank a slurry of black raspberry powder (20 g in 100 ml drinking water) 3 times/day for 1-9 weeks. There was an increase in plasma concentration of GM-CSF) and decrease in IL-8 in patients receiving the berry product for more than 10 days. These alterations were found to be

correlated with beneficial changes in markers of proliferation and apoptosis observed in colorectal tissue taken within the same week (Mentor-Marcel et al., 2012). Based upon this study, plasma concentrations of GM-CSF and IL-8 may serve as non-invasive indicators to monitor tissue response to berry-based interventions for colorectal cancer.

BIOCHEMICAL MECHANISMS OF BERRY PHYTOCHEMICALS

Epidemiological studies have demonstrated an inverse association between the intake of antioxidants from fruits and morbidity and mortality from oxidation-linked diseases, such as cancer, cardiovascular diseases and diabetes (Scalzo et al., 2005). The exact mechanisms of action of the berry phytochemicals remain largely unexplained and are currently being evaluated by various research groups. The complementary and/or synergistic effects resulting from various phytochemicals found in berries might be responsible for their wide range of observed biological properties (Seeram, 2008b). Antioxidant mechanisms have been proposed, especially in the context of cancer, cardiovascular health and age-related degenerative diseases. For inflammation and cancer in particular, modulation of carcinogenesis, phytochemical interactions with vital proteins, signal transduction pathways, pathogen binding and regulation of cancer cell proliferation, immortality and metastasis have also attracted significant attention (Pappas and Schaich, 2009). The mechanism of action of berry components is summarized in Fig. 2.

Oxidative stress caused by a cellular excess of reactive oxygen and nitrogen species have been involved in many processes linked to carcinogenesis such as cell

transformation, proliferation, apoptosis resistance and metastasis by inducing genetic alterations, including DNA damage, mutations, epigenetic changes, or genomic instability. Berry phenolic phytochemicals can affect oxidation stress-related diseases like cancer, by functioning as effective antioxidants through their ability to quench free electrons from biological systems with the phenolic ring and hydroxyl substituents. Berry 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 (Duthie, 2007). Berries of several cultivars of Ribes, Rubus, and Vaccinnium genera have been reported to exhibit significant radical-scavenging activities on chemically-generated superoxide radicals as well as exert inhibitory activity towards the enzyme xanthine oxidase (Costantino et al., 1992). Antioxidant evaluation based on hydrogen-donating ability of antioxidants in a simple *in vitro* model may not indicate their activity in the presence of an oxidizable lipid/protein environment. Berry phenolics, such as anthocyanins, ellagitannins, and proanthocyanidins from raspberry, bilberry, lingonberry, and black currant, were found to be potent antioxidants toward protein and lipid oxidation in a Lactalbumin−Liposome oxidation system (Viljanen et al., 2004). Out of the 4 berries studied, lingonberry and bilberry phenolics followed by black currant and raspberry phenolics exhibited the best antioxidant protection toward lipid oxidation while bilberry and raspberry phenolics exhibit the best overall antioxidant activity toward protein oxidation. Various strawberry extracts containing anthocyanin showed gastroprotective effects against ethanol-induced gastric damage, probably due to their ability to maintain the cell membrane

integrity, reduce the free radical-dependent lipid peroxidation, and preserve and/or activate endogenous antioxidant defense enzymes (Alvarez-Suarez et al., 2011).

Chronic inflammation is associated with the development of diseases like cancer and diabetes. Whole black raspberries have been shown to inhibit mediators of inflammatory processes, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), nuclear factor-kappa B (NF-κB), and various pro-inflammatory cytokines such as granulocyte macrophage colony stimulating factor (GM-CSF), and interleukin-6 (IL-6) and IL-8 (Chen et al., 2006a; Mentor-Marcel et al., 2012; Sardo et al., 2013; Wang et al., 2009). An extract of black raspberries was shown to inhibit benzo(a)pyrene diol epoxide-induced transactivation of NF-κB and activator protein-1 in cultured JB-6 mouse epidermal cells (Huang et al., 2002), and a subsequent study indicated that most active constituents in the extract were the anthocyanins, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside (Hecht et al., 2006). Anthocyanin- and proanthocyanidin-enriched fractions present in blueberry were found to inhibit lipopolysaccharide (LPS)-induced inflammatory responses in mouse macrophages via the NF-κB-mediated pathway (Johnson et al., 2013). Cranberry extract was found to suppress the inflammatory symptoms induced by *Escherichia coli* infections through the inhibition of COX-2 activity (Huang et al., 2009). Cranberry extract also inhibited the NF-κB transcriptional activation in human T lymphocytes, and inhibited the release of IL-1β, IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α) from LPS-stimulated human peripheral blood mononuclear cells (Huang et al., 2009).

Several studies have demonstrated anitmutagenic and anticarcinogenic properties of phenolic phytochemicals, such as ellagic acid (Mass et al., 1991; Stoner and Mukhtar, 1995), caffeic acid, chlorogenic acid, ferulic acid, cinnamic acid, coumaric acid (Yamada and Tomita, 1996), gallic acid, catechin (Mitscher et al., 1993), quercetin (Lu et al., 2006), and resveratrol (Bishayee, 2009; Jang et al., 1997). Mutagenesis is an essential and early event in the carcinogenic process. The anticarcinogenic functions of phenolic phytochemicals may be due to their ability to act as antimutagens. Phenolic phytochemicals from berries can also interfere by inactivating carcinogens, or inhibiting the expression of mutant genes to prevent the development of malignant tumors. Ellagic acid was found to inhibit the metabolism and DNA binding of the carcinogens, *N*-nitrosomethylbenzylamine (NMBA) in cultured rat esophagus (Mandal et al., 1988) and of benzo(a)pyrene in cultured mouse and human lung tissues (Teel et al., 1985; 1986). Later, ellagic acid was found to inhibit hepatic phase I enzymes in liver and induce phase II enzymes in both liver and esophageal mucosa (Ahn et al., 1996). Black raspberry phytochemicals, such as cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside and ellagic acid inhibited hepatic and extrahepatic carcinogen metabolism (Reen et al., 2006). The phenolic compounds from cranberries were found to induce the phase II xenobiotic detoxification enzyme quinone reductase (QR), which is capable of metabolically detoxifying carcinogens (Caillet et al., 2012). 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is an important marker of oxidative DNA damage, which results from free radical damage to guanine, and is highly mutagenic and has been

implicated in cancer development. Urinary levels of 8-OHdG were decreased more than 80% in animals treated with black raspberry extract (Harris et al., 2001).

Helicobacter pylori is a causative agent of gastritis, and untreated infection may lead to gastric cancer (Shanks and El-Omar, 2009). Cranberry nondialyzable materials have been found to inhibit the sialic acid-specific adhesion of antibiotic-resistant and nonresistant *H. pylori* to human gastric mucosa, a critical step in gastric ulcer development, at concentrations that is likely to be achieved after the consumption of cranberry juice cocktail (Shmuely et al., 2004). Similarly high molecular weight polysaccharides from black currant seed extracts were reported to inhibit adhesion of *H. pylori* to human gastric mucosa (Lengsfeld et al., 2004). Nohyenk et al. (2006) have reported that phenolic extracts (1 mg/ml concentration) from cloudberry, bilberry, black current, raspberry and strawberry could inhibit the growth of *H. pylori*.

CONCLUSIONS AND FUTURE DIRECTION

There is a substantial amount of both preclinical and clinical data supporting the concept that berries and some of their individual components have chemopreventive efficacy for cancers of the oral cavity, esophagus and colon. It seems likely that berries would also exhibit preventative effects against stomach cancer and this is most certainly an area for future investigation. Mechanistic studies clearly indicate that berries and many of their constituents prevent cancer at the initiation stage of tumor development by their ability to influence carcinogen metabolism and DNA adduct formation and to scavenge free radicals and reduce oxidative damage to DNA. Undoubtedly, this

reduces mutagenesis in key genes involved in tumor development including cellular oncogenes and tumor suppressor genes. Berries and their components also protectively modulate multiple cellular functions commonly associated with the promotion/progression stages of tumor development, such as proliferation, differentiation, apoptosis, inflammation, angiogenesis, metabolism, motility and tissue invasion. Numerous studies described here have documented the ability of berry constituents to alter the expression levels of genes associated with all of these cellular functions and their associated signaling pathways in a protective manner. The more recent observations that berries reduce the levels of DNA methyltransferases in premalignant and malignant cells leading to demethylation of important suppressor genes involved in the Wnt signaling pathway is of particular interest for the esophagus and colon since Wnt signaling is often aberrant in tumors of these organs. Nevertheless, there remains a number of unanswered questions as to how berry constituents actually influence the expression levels of these genes. Do they function solely as oxidative radical scavengers and influence the effects of oxidative radicals on cell signaling? Do they affect the receptor status of different ligands that influence cell proliferation, differentiation and apoptosis? Or do they enter cells and bind to promoter regions of genes that code for regulatory proteins or bind to enzymes and influence their activities? It seems probable that berry constituents will be found to act through all of these mechanisms and in a host of as yet unidentified mechanisms.

Some of the most active compounds in berries are the polyphenolic constituents. Most polyphenols act as antioxidants and they probably influence numerous cellular

functions through their ability to bind to cellular macromolecules such as proteins and DNA. For example, ellagic acid was found to accumulate in intestinal epithelial cells in vitro and to bind covalently to proteins and non-covalently to DNA (Whitley et al., 2003). A common question is how are these compounds so effective when many of them have poor bioavailability? Pharmacokinetic studies with berry anthocyanins and ellagic acid have shown that the uptake of these compounds into the blood is usually less than 1% of the administered dose (He et al., 2006; Stoner et al., 2005). Undoubtedly, a considerable portion of the chemopreventive activity of these compounds can be attributed to their localized metabolism by oral, esophageal and colonic epithelium. In addition, the microflora in the oral cavity, esophagus and colon are undoubtedly capable of metabolizing these compounds and potentially producing metabolites that exhibit chemopreventive activity. In that regard, the microbial milieu in the saliva of humans is capable of metabolizing black raspberry anthocyanins to their corresponding glucuronides (Mallery et al., 2011). In addition, a pharmacokinetic study showed that about 70% of the cyanidin type anthocyanins found in black raspberries are converted to protocatechuic acid (PCA), a simple polyphenol with antioxidant, antimutagenic and anticarcinogenic effects (Peiffer et al., 2014). PCA exhibits chemopreventive activity in the rat colon and liver (Mori et al., 1997). We have shown that PCA is almost as active in preventing the development of NMBA-induced esophageal papillomas in rats as whole black raspberries and black raspberry anthocyanins (added to the diet in the same concentration as present in whole black raspberries) (Peiffer et al., 2014). PCA was tested at a dietary concentration that represented the conversion of 70% of the

anthocyanins to PCA. These results suggest that PCA may be responsible for a substantial part of the chemopreventive activity of black raspberries and their component anthocyanins and is worthy of additional evaluation for chemoprevention of esophagus, colon and other cancer types. In addition, PCA meets the criteria of an ideal chemopreventive agent because, unlike the anthocyanins, it is stable, easily synthesized and available commercially at relatively low cost. Finally, PCA appears to elicit little or no toxicity at dietary concentrations that elicit chemopreventive effects.

The fiber fraction of berries usually represents about 30-45% of the dry weight of the berry. In one study, the fiber fractions of black raspberries, strawberries and blueberries were found to be nearly as active in preventing the development of esophageal tumors in rats as the whole berries (Wang et al., 2010). The activity in these fractions was probably not due to their content of ellagitannins because the fiber fraction of blueberries contained only trace amounts of ellagitannins and it was equally as effective in preventing esophagus cancer as the fiber fractions from black raspberry and strawberry. Fiber is composed of cellulose, hemicellulose, pectin, lignans and other components. Studies have shown that the fiber in foods is converted to short-chain fatty acids such as butyrate, propionate and lactate by the enteric bacteria and butyrate is chemopreventive for colon cancer (McIntyre et al., 1993). Additional studies are warranted to identify the bioactive compounds and their metabolites from fiber because fiber may be the “common denominator” responsible for the chemopreventive activity of all berry types irrespective of their contents of anthocyanins, phenolic acids and other bioactives.

Human clinical trials demonstrating the chemopreventive effects of berry preparations have focused mainly on black raspberries. However, it is likely that other berry types may also be effective for chemoprevention in humans. Strawberry powder was equally as effective as black raspberry powder in preventing esophageal tumorigenesis in rats (Carlton et al., 2001), and it was remarkably effective in regressing mildly dysplastic lesions in the esophagus of Chinese subjects when consumed for six months at a concentration of 60 g/day (Chen et al., 2012). This may well be the case for other berry types and, importantly, berry types that are more commercially available to the public than black raspberries. So far, the focus of human trials in digestive tract cancers has been that of evaluating the ability of berry formulations to regress dysplastic lesions (oral cavity and esophagus) or polyps (colon, rectum). While this is worthwhile, other objectives could also be investigated such as; a) determine the ability of berry constituents to reduce tobacco carcinogen and oxidative radical damage in the oral cavity of tobacco smokers, b) evaluate the efficacy of berry constituents to enhance the efficacy of chemotherapeutic drugs and radiation for cancer treatment; c) determine whether berries have efficacy for reducing risk for recurrent disease in patients who have been treated for digestive tract cancers; and d) evaluate whether consistent berry use provides a survival advantage in patients who have terminal digestive tract cancers. Anecdotal evidence from patient reports over the years strongly suggests that berry use improves quality of life and may provide a survival advantage in terminal cancer patients but this requires rigorous scientific investigation and proof. Indeed, studies with berry formulations against digestive tract cancers have just begun!

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Table 1: *In vitro* anticarcinogenic effects of berry phytochemicals in GI cancers.

Berry extract, fraction or component	Cellular effects	Mechanism of action	Concentration	References
Oral				
Total polyphenol fraction of cranberry extract	Inhibited the proliferation of CAL-27 and KB cells		6.5 μg/ml; 48 h	Seeram et al., 2004
Blackberry, black and red raspberry, blueberry, cranberry, and strawberry extracts	Exerted antiproliferative effects against CAL-27 and KB cells		25-200 μg/ml; 48 h	Seeram et al., 2006
Black raspberry extracts, fractions and components	Exhibited growth-inhibitory effects in SCC-83-01-82 and 83-01-82CA cells	↓Cyclin A; ↓cyclin B1; ↓cyclin D1; ↓cdc2; ↑$p21^{waf1/cip1}$	1-15 μM; 2 days	Han et al., 2005
Freeze-dried black raspberry ethanol extract	Suppressed the proliferation of human oral squamous cell carcinoma cells	↑Apoptosis; ↑terminal differentiation; ↓VEGF; ↓iNOS	10-100 μg/ml; 24-72 h	Rodrigo et al., 2006
Strawberry crude extract and phenolic compounds	Inhibited the growth of CAL-27 and KB cells	Antioxidant mechanism	250 μg/ml (crude extract) and 100 μg/ml (pure compounds); 48 h	Zhang et al., 2008
Esophagus				
Cranberry proanthocyanidin-rich extract	Showed in vitro anticancer effect against SEG-1 cells	↑Apoptosis; cell cycle arrest	12.5-400 μg/ml; 24-96 h	Kresty et al., 2008

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Freeze-dried black raspberry and anthocyanins	Displayed growth inhibition of highly tumorigenic RE-149DHD rat esophageal cells	↑Apoptosis; ↑caspase-3; ↑caspase-7; ↓COX-2; ↓iNOS	10-100 µg/ml; 4 days	Zikri et al., 2009
Stomach				
Black currant, raspberry, cranberry and gooseberry juice	Exerted antiproliferative effects in AGS cells		50 µl/ml; 48 h	Boivin et al., 2007
Red raspberry extract	Reduced the survival of AGS cells		5-10%; 48 h	God et al., 2010
Malvidin	Inhibited the proliferation of AGS cells	↑Apoptosis; ↑caspase-3; ↑Bax/Bcl-2; ↑p38 kinase; ↓ERK	20-200 µM; 48 h	Shih et al., 2004
Malvidin, Pelargonidin	Exerted antiproliferative effects against AGS cells		12.5-200 µg/ml; 48 h	Zhang et al., 2005
Liver				
Strawberry extracts	Induced antiproliferative activities against HepG2 cells		5-75 mg/ml; 96 h	Meyers et al., 2003
Blueberry fractions	Retarded the growth of HepG2 cells			Yi et al., 2006
Anthocyanin-rich black currant extract	Suppressed the growth of HepG2 cells		0.1-4 mg/ml; 24 h	Bishayee et al., 2010
Quercetin, ursolic acid and 3,5,7,3′,4′-penahydroxyflavonol-3-*O*-β-D-glucopyranoside	Inhibited the proliferation of HepG2 cells	Antioxidant mechanism	41-87 µM (EC_{50}) 96 h	He and Liu, 2006
Delphinidin	Showed growth inhibition of HepG2 cells	↑Apoptosis; ↑caspase-3; ↑Bax; ↓Bcl-2; ↑c-Jun; ↑p-JNK	10-200 µM; 24h	Yeh and Yen, 2005

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	Triggered growth retardation of HepG2 cells	Autophagy	100 μM; 48-120 h	Feng et al., 2010
Colon				
Bilberry extract	Induced growth inhibition of HCT116 human colon cancer cells		1-4 mg/ml; 24, 48 h	Katsube et al., 2003
	Exhibited cytotoxicity against HT-29 colon cancer cells	↓DNA damage; ↓ROS; ↑GSH	5–500 μg/ml; 1, 24 h	Schantz et al., 2010
Bilberry, chokeberry and elderberry anthocyanin-rich extracts	Retarded the growth of HT-29 cells		10-100 μg anthocyanin/ml; 24-72 h	Zhao et al., 2004; Jing et al., 2008
Chokeberry anthocyanin-rich extract	Displayed growth-inhibitory effect in HT-29 cells	Cell cycle arrest; ↑$p21^{WAF1}$; ↑$p27^{KIP1}$; ↓cyclin A; ↓cyclin B; ↓COX-2	50 μg anthocyanin/ml; 24-72 h	Malik et al., 2003
Cranberry presscake extract	Exhibited antiproliferative activity against HT-29 cells		100-500 mg/ml; 4 d	Ferguson et al., 2004
Total cranberry extract and its phytochemical-rich fractions	Inhibited the proliferation of HT-29, HCT-116, SW480 and SW620 colon cancer cells		50-200 μg/ml (extract) & 6.5-78.8 μg/ml (fractions); 48 h	Seeram et al., 2004
Blackberry, black and red raspberry, blueberry, cranberry, and strawberry extracts	Displayed inhibition of proliferation of HT-29 and HCT-116 cells	↑Apoptosis	25-200 μg/ml; 48 h	Seeram et al., 2006
Black chokeberry, lingon berry and raspberry extracts	Retarded the proliferation of HT-29 cells		0.025-0.5%; 24 h	Olsson et al., 2004
Blueberries	Inhibited proliferation of HT-29 and	↑Apoptosis	50-10,000	Yi et al., 2005

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phenolic extracts and fractions	Caco-2 cells		µg/ml; 48 h	
Anthocyanin-rich fraction from blueberry	Induced programmed cell death in HT-29 cells	↑Caspase-3; ↓QR; ↓GST	50-150 µg/ml; 6h	Srivastava et al., 2007
Berry phenolic extracts	Inhibited the proliferation of HT-29 cells	↑Apoptosis; ↑$p21^{WAF1}$; ↑Bax	5-60 mg/ml; 24 h	Wu et al., 2007
Anthocyanin-rich extract from Chinese blueberry	Repressed the proliferation of COLO205 human colorectal cancer cells	↑Apoptosis	50-250 µg/m; 24 h	Zu et al., 2010
Strawberry extracts	Displayed antiproliferative activity in HT-29 cells		0.025-0.5%; 24 h	Olsson et al., 2006
Extract from strawberries treated with essential oils	Exhibited inhibition of HT-29 cell growth		3 mg/ml; 1-4 days	Wang et al., 2007
Strawberry crude extract and phenolic compounds	Inhibited the proliferation of HT-29 and HCT-116 cells	Antioxidant mechanism	250 µg/ml (crude extract) and 100 µg/ml (pure compounds); 48 h	Zhang et al., 2008
Strawberry, arctic bramble, cloudberry and lingon berry extracts	Showed antiproliferative effects in CaCo-2 cells		25-75 µg/ml; 72 h	McDougall et al., 2008
Raspberry extract	Failed to exhibit cytotoxic effect in HT-29 cells, but inhibited the invasion of HT-115 cells	↓cells in G_1 phase	3.125-50 µg/ml; 24 h	Coates et al., 2007
Anthocyanins from black raspberry	Decreased the proliferation of HCT-116, CaCo-2 and SW480 cells	↑Apoptosis; ↓β-catenin; ↓c-Myc; ↓DNMT1; ↓DNMT3B	0.5, 5, 25 µg/ml; 3 days	Wang et al., 2013
Red raspberry extract	Reduced the survival of LoVo human colon cancer cells		5-10%; 48 h	God et al., 2010

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Black currant juice	Suppressed the proliferation of CaCo-2 cells		50 μl/ml; 48 h	Boivin et al., 2007
Black currant press residue extract	Curtailed the proliferation of CaCo-2, HCT-116 and HT-29 cells	↑Apoptosis (HT-29)	20-125 μg/ml; 24 h	Holtung et al., 2011
Cyanidin	Inhibited the growth of HT-29 cells		10, 100 μM; 72 h	Briviba et al., 2001
Delphinidin	Inhibited the proliferation of Caco-2 cells	↑Apoptosis; ↓cells in G_1 phase	50-200 μM; 24 h	Lazze et al., 2004
Delphinidin	Suppressed the HCT-116 cells	↑Apoptosis; ↑caspase-3, -8 and -9; ↑Bax; ↓Bcl-2; ↓IKKα; ↓IκBα; ↓NF-κBp65	30-240 μM; 48 h	Yun et al., 2009
Cyanidin, Delphinidin	Exerted cytotoxic effects in both LoVo and LoVo/ADR cells	↓GR; ↓GSH; ↑ROS	0.78-100 μM; 68 h	Cvorovic et al., 2010
Malvidin, Pelargonidin	Displayed antiproliferative effects of HCT-116 cells		12.5-200 μg/ml; 48 h	Zhang et al., 2005
(−)-gallocatechin, (−)-gallocatechin gallate	Inhibited the growth of HCT-116 cells		10-100 μM; 48 h	Seeram et al., 2003
Ellagic acid	Showed antiproliferative and antimigratory activities against Caco-2 cells	↑Apoptosis; ↓pro-MMP-2; ↓pro-MMP-9; ↓VEGF	1-100 μg/ml; 6 h	Losso et al., 2004
	Inhibited the proliferation of Caco-2 cells	↑Apoptosis; ↓cyclin A; ↓cyclin B1; ↑cyclin E; ↑caspase-3; ↑caspase-9	1-30 μM; 24-72 h	Larrosa et al., 2006

Table 2: *In vivo* anticarcinogenic effects of berry phytochemicals in GI cancers.

Berry extract, fraction or component	Chemopreventive or therapeutic effect	Mechanism of action	Dose/duration	Routes	References
Oral					
Freeze-dried black raspberry powder	Exhibited chemoprevention of DMBA-induced oral cavity tumors in male Syrian Golden hamsters	↓DNA adducts	5,10%; 12-13 weeks	Diet	Casto et al., 2002
Esophagus					
Freeze-dried strawberry	Reduced the multiplicity of tumors induced by NMBA in male F344 rats	↓O^6-meGua	5, 10%; 24 weeks	Diet	Stoner et al., 1999
	Decreased the multiplicity of esophageal tumors initiated by NMBA in male F344 rat	↓O^6meGua	5, 10%; 20, 32 weeks	Diet	Carlton et al., 2001
Freeze-dried black raspberry	Exerted chemoprevention of NMBA-induced esophageal tumorgenesis in F344 rats during initiation and promotion phases	↓O^6meGua; ↓PCNA	5, 10% or 0.25 mg/kg; 30 or 35 weeks	Diet	Kresty et al., 2001
	Reduced the multiplicity of esophageal tumors induced by NMBA in male F344 rats	↓COX-2; ↓iNOS; ↓c-Jun; ↓PGE_2; ↓VEGF	5%; 25 weeks	Diet	Chen et al. 2006a; 2006b
	Failed to reduce the incidence, number and size of NMBA-induced pappilimas in male F344 rats		5-20%; 7 weeks	Diet	Stoner and Aziz, 2007
	Reversed histopathological alterations in esophagi of male F344 rats exposed to NMBA	Modulation of genes involved in phase I and II metabolism, apoptosis, cell cycling and	5%; 3 weeks	Diet	Stoner et al., 2008b

		angiogenesis			
	Attenuated NMBA-induced esophageal tumorigenesis in male F344 rats	↑Apoptosis; ↓Bcl-2; ↑Bax; ↓Ki-67; ↓COX-2; ↓PGE_2; ↓NF-κB; ↓CD34; ↓CD45	5%; 30 weeks	Diet	Wang et al., 2009
	Inhibited post-initiation phase of NMBA-induced esophageal carcinogenesis in male F344 rats	Modulations of genes and proteins involved in proliferation, apoptosis, inflammation, angiogenesis and metastasis	5%; 30 weeks	Diet	Wang et al., 2011a
Freeze-dried back raspberry, blackberry and strawberry	Inhibited the numbers of papillomas in NMBA-treated F344 rats	↓O^6meGua; ↓PCNA; ↓COX-2; ↓PGE_2; ↓iNOS	5, 10%; 18, 25 weeks	Diet	Stoner et al., 2006
Black and red raspberry, blueberry, strawberry, açaí and wolfberry	Reduced the incidence and multiplicity of NMBA-induced tumors in male F344 rats	↓IL-5; ↓GRO/KC; antioxidant mechanism	5%; 35 weeks	Diet	Stoner et al., 2010
Ellagic acid	Decreased the preneoplastic lesions, neoplastic lesions and number of NMBA-induced esophageal tumors in male F-344 rats		0.4, 4 g/kg; 20, 27 weeks	Diet	Mandal and Stoner, 1990
	Decreased the multiplicity and size of tumors induced by NMBA in male F-344 rats		4 g/kg; 25 weeks	Diet	Daniel and Stoner, 1991
	Reduced tumor incidence in male F344 rats exposed to NMBA		400, 4000 ppm; 25 weeks	Diet	Siglin et al., 1995
	Lowered the incidence and		0.4, 4.0 g/kg;	Diet	Stoner and

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	multiplicity of NMBA-induced tumors in male F344 rats		20, 27 weeks		Morse, 1997
Liver					
Anthocyanin-rich black currant extract	Inhibited DENA-initiated and PB-promoted hepatocarcinogenesis in male rats	↑Apoptosis; ↓PCNA; ↑Bax; ↓Bcl-2; ↓COX-2; ↓HSP70; ↓HSP90; ↓NF-κB; ↓INOS; ↓3-NT; ↓Nrf2	100, 500 mg/kg; 22 weeks	Diet	Bishayee et al. 2011; 2013; Thoppil et al., 2012
Small and large intestines					
Anthocyanin-rich bilberry extract or cyanidine-3-*O*-glucoside	Decrease the total numbers of intestine adenomas in APC^{Min} mice		0.03-0.3%; 12 weeks	Diet	Cooke et al., 2006
Anthocyanin-rich bilberry and chokeberry extracts	Decreased the number of total and large ACF in AOM-treated male F344 rats	↓PCNA; ↓COX-2	3.85 g anthocyanin/kg; 14 weeks	Diet	Lala et al., 2006
Freeze-dried bilberry, lingonberry and cloudberry	Inhibited the formation of intestinal adenoma in *Min*/+ mice	↓Cyclin D1; ↓β-catenin; ↓Ada; ↓5′-NT; ↓EP4	1,564 mg/kg; 10 weeks	Diet	Mutanen et al., 2008
Blueberry, blackberry and cranberry products	Reduced the total numbers of AOM-induced ACF in F344 male rats	↑GST	5-20%; 17 weeks	Diet, drinking water	Boateng et al., 2007
Freeze-dried white currant	Decrease the number and size of intestinal adenomas in *Min*/+ mice	↓β-catenin; ↓NF-κB	10%; 10 weeks	Diet	Rajakangas et al., 2008
Freeze-dried black raspberry	Suppressed AOM-induced colon carcinogenesis in male F344 rats	↓8-OHdG	2.5-10%; 9, 33 weeks	Diet	Harris et al., 2001
	Ameliorated DDS-induced ulcerative colitis in male C57BL/6J mice	↓IL-1β; ↓TNF-α; ↓COX-2; ↓PGE_2; ↓p-IκBα	5, 10%; 7-14 days	Diet	Montrose et al., 2011
	Inhibited DDS-induced colonic	↓NF-κB p65; ↓β-catenin;	5%	Diet	Wang et al.,

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	ulceration in male C57BL/6J mice	↓c-Myc; ↓DNMT3B; ↓HDAC1; ↓HDAC2; ↓MBD	28 days		2013b
	Decreased colonic ulceration in IL-10 knockout male mice	↓β-catenin; ↑wif1; ↑sox17; ↑qki; ↑dkk2; ↑dkk3; ↓wnt3a	5%; 8 weeks	Diet	Wang et al., 2013c
	Lowered tumor incidence and multiplicity in *Apc*1638 +/- and *Muc*2-/- mice	↓PCNA; ↓β-catenin; ↓COX-2; ↓IL-1; ↓IL-6; ↓IL-10; ↓TNF-α; ↑E-cadherin	10% w/w; 12 weeks	Diet	Bi et al., 2010

Legends to figures

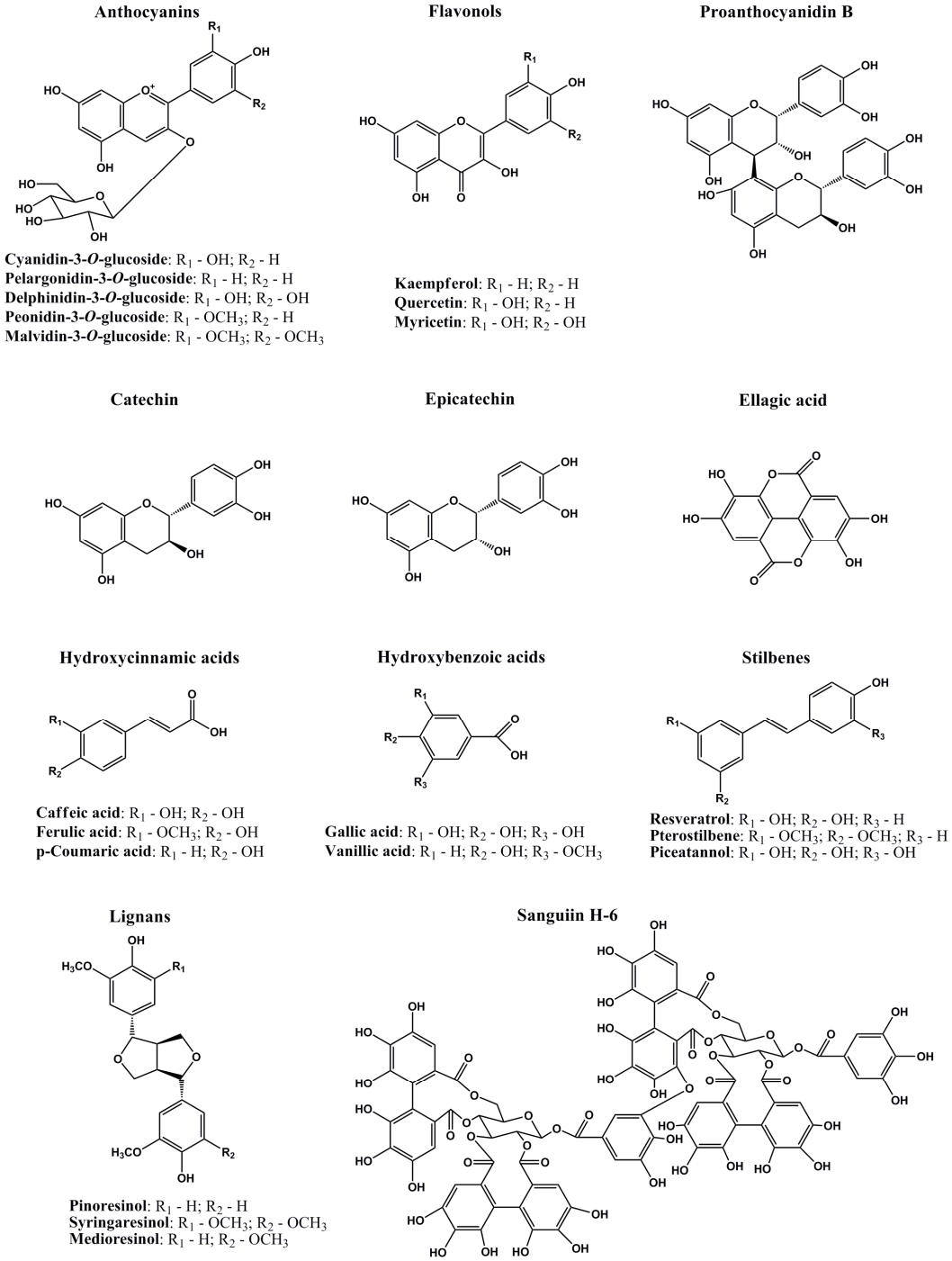

Fig. 1. Chemical structures of bioactive phytoconstituents present in berries.

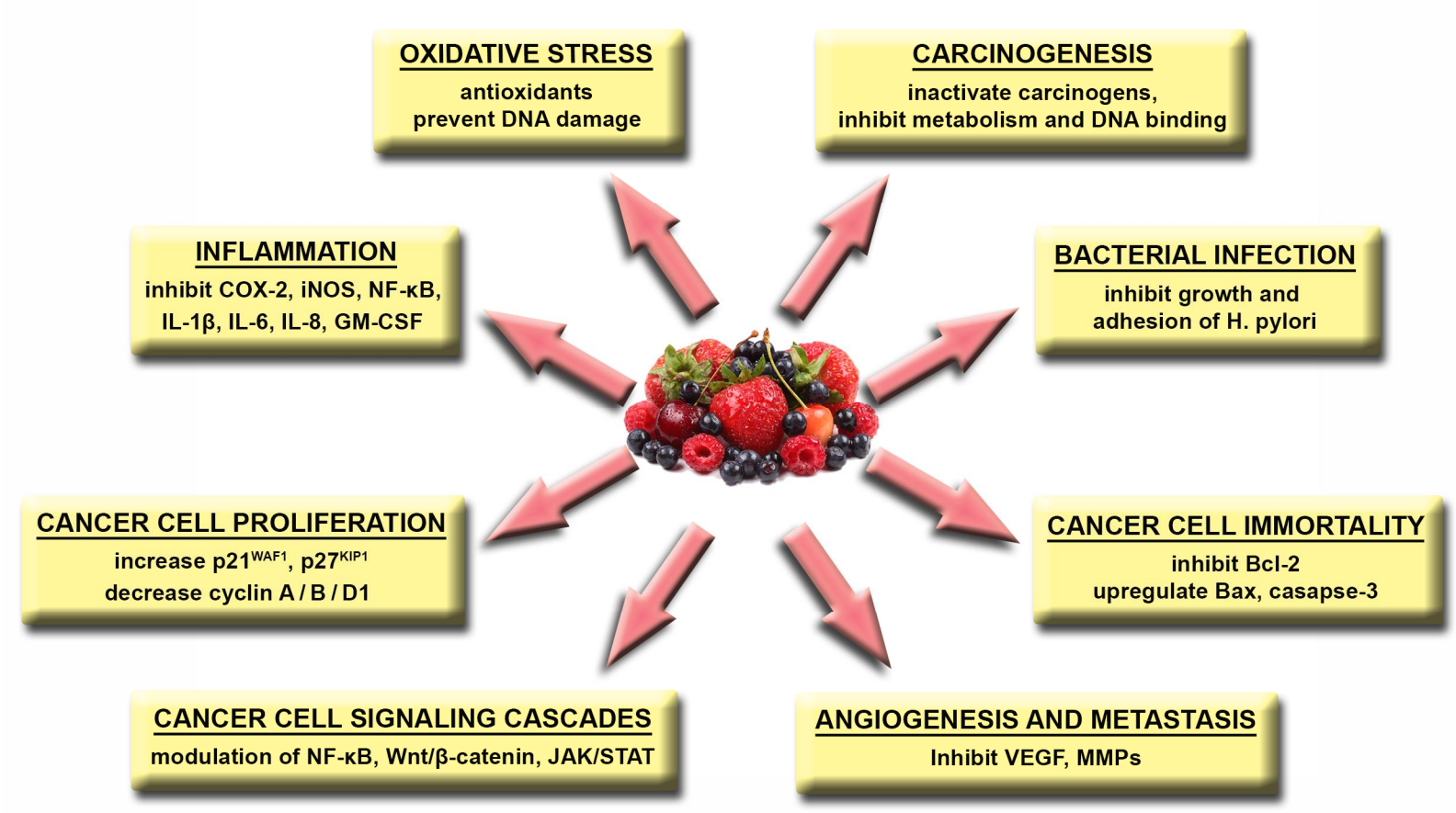

Fig. 2. Various cellular mechanisms and targets of berry components.