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Berry chemoprevention: do berries decrease the window of opportunity for tumourigenesis

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Abstract

In addition to smoking and UV exposure, lifestyle factors such as diet, nutrition and physical activity, have been shown to play a significant role for many cancers. It is estimated that up to 50% of some cancer types are preventable; many through lifestyle and dietary changes with the presence or absence of certain dietary components strongly associated with an increased or decreased risk. Here we summarise the work that has been performed with polyphenols, with a focus on those derived from black raspberries. These have been extensively studied for the prevention and treatment of a variety of conditions and diseases. Here we focus on their use for the prevention or treatment of specific cancer types and the impact they have on biological systems. The aim is to highlight the need to improve our understanding of how the environment impacts upon the normal biological processes that affect health and disease, thereby, enabling us to implement smarter prevention and treatment measures.

1. Introduction

The global increase in the incidence and mortality of all cancer types is leading to an escalating burden on global economics and healthcare[1]. The reasons behind this are somewhat complex, but reflect a rapid population growth, an increased ageing population and alterations to the prevalence and distribution of risk factors attributed to the disease[1]. Lifestyle factors, such as diet, obesity, and

socioeconomic status have a profound impact on cancer incidence. Combined, these highlight an increasing demand for strategies for cancer prevention, as there is substantive evidence that diet, nutrition and physical activity play fundamental roles in cancer and disease prevention[2]. Over the last two decades, a substantial amount of research has focused on the chemopreventative and anti-tumourigenic properties of dietary polyphenols, such as those found in many fruits and vegetables. In this review we will present the current position on lifestyle factors and cancer, the utilization of polyphenols by the body, summarise the data on polyphenols and specific tumour types, and how they influence the epigenome, microbiome and immunity.

1.1 Established causes of cancer

There are over 100 different types of cancer, which is complicated further by the several sub-classifications within each cancer[2]. Although the exact mechanisms and behaviours behind each cancer is not currently understood, the complex relationship between cancer, host, nutrition and physical activity are being explored. At a molecular level cancer arises from an accumulation of DNA damage and as a result of abnormal and uncontrollable cell division. There are several factors identified under the classic 'hallmarks' that can compromise normal cell division[3, 4], these can be broadly divided into two groups; 'endogenous' and 'exogenous' factors[2, 3], Figure 1.

1.2 'Endogenous' Factors

Endogenous factors arise from processes within the individual. Less than 10% of cancers are attributed to inherited genetic mutations often affecting a tumour suppressor gene, for example, patients with familial adenomatous polyposis (FAP), inherit a mutated *Adenomatosis polyposis coli* (*APC*) gene and are predisposed to colorectal cancer (CRC)[5]. Other endogenous factors include imbalances in hormonal and metabolic pathways, chronic inflammation associated with DNA damage through genetic and epigenetic modifications and oxidative stress[3].

1.3 'Exogenous' Factors

Exogenous factors are commonly termed as lifestyle factors. These include, carcinogens from smoking, excessive alcohol consumption, infectious agents: such as liver cancer from hepatitis B and C infections and *Helicobacter pylori* a cause of stomach cancer[2]; ionizing radiation from ultraviolet (UV) light and influences from nutrition, body fatness and physical activity[2]. The relationship between diet or physical activity and cancer are specific for certain cancers, such as the relationship between CRC and diets consisting of red and processed meats[6]. However, it is often the case that several exogenous exposures relating to diet and physical activity, are attributed to numerous cancer types[2].

In the UK around 4 in 10 cancer cases a year are linked to exogenous factors. The largest single cause of cancer is smoking, the predominant cause of lung cancer. Body fatness and obesity are

estimated to contribute to 6.3% of cancer cases. UV radiation and occupational risks make up 3.8% of UK cancer cases. Dietary factors have significant roles in the aetiology of many cancers, particularly CRC, excessive alcohol consumption, insufficient fibre intake and infections are attributable to 2-4% of cases[7]. To fully understand the chemopreventative mechanisms underpinning nutritional factors in cancer, both large-scale epidemiological and preclinical studies are necessary. One area of intense research has focused on the protective role of bioactive phytochemicals, in particular polyphenols.

1.4 Dietary polyphenols

Polyphenols are naturally occurring compounds enriched in fruits, vegetables and cereals and are a major source of antioxidants in the human diet. They are secondary metabolites in plants that aid in protecting against UV radiation and aggression by pathogens[8]. For many years plant-derived nutritional polyphenols have been investigated for their anti-tumourigenic[9] properties, protection against cardiovascular disease, diabetes, neurodegenerative disease[10], anti-inflammatory[11], anti-thrombotic[12], and anti-viral[13] properties.

These antioxidants prevent damage to cells by scavenging and neutralising free radicals and reactive oxygen species (ROS) thereby reducing oxidative stress. Although ROS are naturally produced within the body from normal oxidative metabolism[14], accumulation of these species can be detrimental. High concentrations of free radicals and ROS can result in extensive DNA, membrane lipid and protein damage which can potentiate the development of cancer[14]. Naturally produced endogenous antioxidants remove free radicals and prevent cellular damage in the body. However, the body heavily relies on exogenous sources of antioxidants, mainly from the diet[15]. The most abundant antioxidants in our food are the polyphenols.

Based upon their chemical structure, nutritional polyphenols can be divided into several groups: the flavonoids, phenolic acids, stilbenes and lignans[12, 16]. The flavonoids can be further divided into six subgroups, flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols (catechins and proanthocyanidins).

1.5 Anthocyanins

The most abundant flavonoid found in fruit and vegetables are the anthocyanins. Anthocyanins are responsible for the variety of colours, most notably red, blue, purple and black, in the fruits, flowers, leaves, stems, and roots of plants[17]. Their bright colours attract animals and insects, aiding pollination and seed dispersal of plants. Additionally, their antioxidant properties defend against extreme UV exposure, pathogens and oxidative stress[18]. In humans, their antioxidant, anti-carcinogenic and anti-inflammatory properties[19] have been widely investigated as a potential treatment for many cancers.

In the USA the estimated daily intake of anthocyanins is around 180 - 215 mg, approximately nine-fold more than other dietary flavonoids which are consumed at about 23 mg/ day[17]. These natural anthocyanins are well represented in our diet as they occur in red, blue and purple produce such as carrots, onions, aubergines, and grapes but they are most abundant in berry fruits (Table 1) [12, 20], having as high as 1480 mg of anthocyanin/ 100 g fresh weight berry[21]. The concentration of anthocyanins in fruits is dependent on the cultivator, growing conditions (e.g. amount and quality of sunlight, soil composition, salinity and temperature) and season[22]. There have been over 400 different anthocyanins identified, but the 6 most common anthocyanidins (the sugar-free counterparts of anthocyanins) are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin, with cyanidin being the most abundant in nature. One berry known for its particularly high anthocyanin content is the black raspberry (BRB) (approximately 607 mg/ 100 g fresh weight) (Table 1). Studies have indicated that BRB anthocyanins have chemoprotective roles[23, 24] and thus may have a potential use as a chemopreventative strategy against cancer. The main anthocyanins in BRBS are cyanidin-3-glucoside, cyanidin-3-sambubioside, cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside and pelargonidin-3-rutinoside[25].

Berry Type	**Scientific Name**	**Anthocyanin Content (mg/ 100g FW)**	**Reference**
Black chokeberry	*Aronia melanocarpa*	307 ~ 1480	[21, 26]
Elderberry	*Sambucus nigra*	332 ~ 1374	[21, 27]
Bilberry	*Vaccinium myrtillus*	300 ~ 808	[27, 28, 29]
Black raspberry	*Rubus occidentalis*	145 ~ 607	[30, 31]
Blueberry	*Vaccinium corymbosum*	63 ~ 430	[28, 29, 31, 32]
Cranberry	*Vaccinium macrocarpon*	19 ~ 360	[28, 29, 31, 32]
Evergreen blackberry	*Rubus laciniatus*	91 ~ 164	[29, 33, 34]
Marionberry	Rubus ursinus	62 ~ 200	[33, 34, 35]

Red raspberry	Rubus idaeus	28 ~ 104	[29, 30, 31, 32]
Strawberry	Fragaria x ananassa	13 ~ 55	[29, 32, 36]
Anthocyanin content (mg/ 100 g fresh weight (FW)) in selected berries.			

Table 1: Anthocyanin content in edible berry fruits

Range of anthocyanin content found in several berry fruits.

1.6 Anthocyanin Metabolism

Anthocyanins have poor bioavailability, often with as little as 0.1% of the ingested amount being detectable in urine[37], but berry metabolites can be detected in urine, faeces, blood and tissues. It is not fully understood why anthocyanins have a low bioavailability and thus this could be seen as a major limitation to the potential efficacy of berry chemopreventative interventions outside of the gastrointestinal tract. However, as ~60% of ingested anthocyanins reach the colon and come into direct contact with the gastrointestinal epithelium it may explain why berries have higher protective roles against gastrointestinal cancers[38]. Anthocyanin metabolism occurs within three distinct regions of the gastrointestinal tract, the stomach, the small intestine and the colon. These regions are distinct from one another due to varying pH and microbial diversities. Anthocyanins are most stable in the stomach due to a low pH (pH 1-2), and it is here intact anthocyanins are absorbed into the circulatory system. Most anthocyanins survive intestinal transit where they eventually get metabolised by the colonic microflora into a plethora of phenolic acids and other degradative products before being absorbed into the circulatory system. Anthocyanins can be sub-classified into sugar-free anthocyanidin aglycones and anthocyanin glycosides. The intestinal microflora hydrolyse the sugar moieties (glycosides) attached to anthocyanins, the higher pH (neutral) in the colon aids in the metabolism of anthocyanin aglycones to phenolic acids[37, 38] (Figure 2).

1.7 Black Raspberries

The black raspberry (*Rubus occidentalis*) is a minor but speciality fruit of eastern North America and shares its name with its western North American relative *Rubus leucodermis*. BRBs contain a range of nutrients and bioactive phytochemicals such as vitamin A, C, and E, folic acid, calcium, selenium, β-sitosterol, fibre, a variety of polyphenols such as ellagic acid, ferulic acid, ellagitannins, most abundantly anthocyanins, and other bioflavonoids[39]. Due to their expansive health benefits and protective properties against many human diseases and cancer, BRBs have been the most studied berry[38]. The chemopreventative properties of BRBs, in particular, their anthocyanin counterparts

have been investigated *in vitro* and *in vivo* in both human and animal models[24] with the best documented studies being for oral, oesophageal and colon cancers[40].

1.8 Chemopreventive Studies of Black Raspberries

1.8.1 Chemopreventive Studies of Black Raspberries in Oral Cancer

Oral cancer is the most common type of head and neck cancer and in 90% of cases it presents as a squamous cell carcinoma (SCC)[41] with strong evidence linking it to exogenous factors such as smoking and excessive alcohol consumption[41-43]. In 2006, a study investigated the effects of freeze-dried BRB ethanol extracts at varying concentrations on cell lines derived from human SCC. The results indicated that the BRB extract had anti-proliferative effects in a dose-dependent manner and could induce apoptosis, terminal differentiation and suppress the translation of VEGF (vascular endothelial growth factor; potentially inhibiting angiogenesis which would aid tumour development)[44].

In a clinical study with oral intraepithelial neoplasia (IEN), a precursor of oral SCC, topical application of a 10% freeze-dried BRB mucoadhesive gel was administered (0.5g) to the tongue four times a day for 6 weeks. The study reported that the lesion grade decreased in 41% of patients, while 23% of patients had an increase in lesion grade and 35% of participants remained at a stable state. Importantly, the study concluded that freeze-dried BRB gel was safe to use as patients enrolled in the clinical trial reported no BRB gel associated toxicities[45]. The BRB-induced reduction in size and grade of oral IEN lesions may in part, be attributed to increased levels of pro-apoptotic and terminal differentiation genes, reduced epithelial levels of the pro-inflammatory proteins cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). However, the study reported significant interpatient variation[46].

The success of these studies led to the development of a multi-centred placebo-controlled trial, investigating the efficacy of the BRB gel over a 3-month period. The previously described dosing regime was utilised in patients with confirmed oral IEN. Compared to placebo treated patients, BRB gel intervention resulted in significant reductions in both lesion size and grade[47]. In a more recent clinical trial, oral cancer patients were treated with dissolvable slow-release BRB troches which equated to a daily dose of 4.3g freeze-dried BRB powder. Treatment with these BRB troches resulted in the reduction of pro-inflammatory and pro-survival genes and reduced biomarker expression of genes dysregulated in oral cancers[48].

Since these clinical trials, substantial work has focused on the development of suitable *in vivo* animal models that better represent carcinogen induced oral dysplasia's in humans, such that the chemopreventative mechanisms of BRBs could be further investigated. In 2012, a novel non-surgical murine model of oral cancer was developed, whereby animals were exposed to an environmental

pollutant and component of tobacco smoke dibenzo[*a,l*]pyrene, and its diol epoxide metabolite. Topical exposure of these carcinogens to the oral cavity of mice resulted in 74% and 100% OSCC in tongue and oral tissues respectively; mediated via the induction of DNA adducts, prerequisites for oral carcinogenesis[49 50]. These mice were fed either a control diet (AIN-93M) or a diet supplemented with 5% BRB powder for 2 weeks prior to the induction of tumourigenesis in the oral cavity. The study reported that administration of a 5% BRB diet prior to carcinogen exposures resulted in fewer DNA adducts and abrogated tumour incidence from 93% to 66%[51]. It was later reported that BRB-mediated reduction in oral mutagenesis could be attributed, in part, to epigenetics and hyper-methylation of genes involved in epithelial-mesenchymal transition (EMT)[52]. These BRB-mediated metabolomic effects in oral cancer are further supported in a study by Knobloch *et al.* (2019), using a chemically-induced model of oral cancer, (4-nitroquinoline-1-oxide)[41], whereby 6-week exposure to a 10% BRB diet inhibited enzymes fundamental for the metabolic pathway of glycolysis, thereby limiting nutrient availability for cancer cells[53].

1.8.2 Chemopreventive Studies of Black Raspberries in Oesophageal Cancer

Worldwide, oesophageal cancer ranks the 7th most common cancer[1]. Oesophageal cancers present as one of the two most common histological subtypes; SSC or adenocarcinoma and is linked to excessive alcohol consumption, tobacco carcinogens or drinking scalding hot mate (a plant-based infusion drunk in parts of South America), whereas adenocarcinomas are often attributed to higher socioeconomic countries where obesity and gastroesophageal reflux disease (GERD) are on the rise[2, 54].

In 2001, *in vivo* studies using the NMBA (N-nitrosomethylbenzylamine) rat model of oesophageal cancer reported that feeding of 5 and 10% lyophilised BRB diet for 2 weeks prior to NMBA exposure and then throughout a 30 week time period, resulted in significant reduction in tumour multiplicity and inhibited the formation of mutagenic DNA adducts[55]. Additional studies with this model have reported that BRB diet reduced rat oesophageal cancer by decreasing DNA adducts (specifically O^6-methylguanine), inhibiting proliferation and dampening down expression of pro-inflammatory enzyme Cox-2[56], reversing oxidative stress[56, 57] and via inhibition of pro-angiogenesis genes such as *Vegf*[58]. BRBs have also been shown to inhibit NMBA-induced oesophageal cancer by inhibiting the Wnt signalling pathway through alleviation of hypermethylation of the Wnt pathway antagonist, Sfrp4 (secreted frizzled related protein 4), and reduction in mRNA levels of DNA methyltransferases, *Dnmt1* and *Dnmt3b*. These BRB-mediated alterations resulted in significant reductions in nuclear β-catenin localisation thus preventing proliferation of rat oesophageal squamous cell papilloma[59].

In 2016, BRBs were used in a phase I pilot study in patients with Barrett's esophagus (BE), a precursor lesion for oesophageal adenocarcinoma (EAC). To determine the long-term tolerability of BRBs in oesophageal cancer, a 6 month pilot study was performed. Based on rodent pre-clinical studies, patients confirmed to have BE were administered lyophilised or freeze-dried BRB powder at 32 and 45g (~1.5 and 2 cups of whole fruit)[40, 55, 56, 60]. Results showed that lyophilised BRBs were generally well tolerated with a few adverse effects including epigastric pain, diarrhoea and constipation, and resulted in increased levels of GST-pi (a marker of detoxification) in over 50% of patients. Suggesting BRBs may help prevent BE progression by detoxification of the mutagenic components involved in gastroesophageal reflux disease[61, 62].

In addition, the chemopreventive properties of BRBs in oesophageal cancer have been linked in part to their effects on inflammation and immune cell trafficking. Exposure to 6.1% BRB diet, a BRB-derived anthocyanin-enriched fraction (3.8 μmol/g) or treatment with protocatechuic acid, a major microbial-generated metabolite of BRB anthocyanins, inhibited NMBA-induced rat oesophageal cancer. Mechanistically, this was found to occur through inhibition of inflammatory genes *Cox-2* and *IL-1β,* reduced infiltration of inflammatory macrophages into dysplastic lesions, limited neutrophil and cytokine accumulation and decreased angiogenesis[63, 64].

Similar results to both of these studies have been reported using human oesophageal microvascular endothelial cells (HEMEC) stimulated with either the pro-inflammatory cytokine tumour necrosis factor-alpha (TNF-α) or IL-1β. HEMEC cells were pre-treated with BRB ethanol extract for 2 hours before activation with TNF-α or IL-1β. BRB ethanol extract had a strong anti-inflammatory effect by inhibiting NF-κB nuclear translocation, reducing COX-2 gene and protein levels in stimulated HEMECs, abolished the pro-angiogenic effects of VEGF by inhibiting cell migration, proliferation, and survival of VEGF-stimulated HEMECs[65].

1.8.3 Chemopreventive Studies of Black Raspberries in Colorectal Cancer

Colorectal cancer (CRC), which encompasses both colon and rectal tumours, is the third most common cancer in men (10% of total cancer cases) and the second most common cancer in women (9.2% of total cancer cases) worldwide[66]. In 2018 alone, it was estimated that there were more than 1.8 million new CRC diagnoses and 881,000 deaths reported[1], highlighting the burden CRC puts on the healthcare infrastructure. CRC incidence displays wide geographical variation globally, with similar patterns seen for both men and women. CRC is often considered a marker of socioeconomic development since it mostly occurs in countries undergoing major development and nutritional transition and in those which have a high human development index[1, 2, 66].

The majority of CRCs are adenocarcinomas (approximately 95%) which occur sporadically, with only a small proportion of CRC cases attributable to inherited genetic mutations (~10%); mainly

FAP and Lynch Syndrome. There is evidence that CRC likely develops through an interplay of endogenous (epigenetic, microbial status, inflammation, immune status) and exogenous factors (diet composition, stress, alcohol consumption, smoking and obesity) which increase mutation rates[67]. In the UK alone it is estimated that around half of CRC cases could have been prevented through lifestyle modifications such as alteration to the diet and reduction in body fatness[2].The combination of damage from carcinogens that directly interact with the intestinal and colonic epithelium, and the mechanical deformation likely underpins the high rate of intestinal stem cell (ISC) division which is required to maintain an intact epithelial barrier. In turn this can lead to increased CRC risk due to a build-up of oncogenic DNA mutations in ISCs[68].

In 2001, a study investigated the protective effects of lyophilised BRBs in the azoxymethane (AOM) rat model of CRC[69]. AOM-induced rodent CRC, occurs via the production of DNA adducts following AOM metabolism, and faithfully models sporadic and hereditary human CRC development sharing similar genetics, histopathological characteristics and defective mismatch repair to the human disease[70]. Twenty-four hours after final treatment with AOM, rats were fed either 0%, 2.5%, 5% or 10% freeze-dried BRB diet for 9 and 33 weeks. The total tumour multiplicity decreased by 42%, 45%, and 71% respectively, in a dose-dependent manner, tumour burden also decreased in a similar fashion but was not found to be significantly different. This demonstrates the ability of BRBs to reduce tumourigenesis in a rodent model of colon carcinogenesis[69].

In 2010, Bi and colleagues performed *in vivo* experiments in two genetic murine models of human CRC to investigate whether BRBs would inhibit tumourigenesis[71]. The two mouse models used were *Apc*1638$^{+/-}$, which develops small intestinal tumours as a result of having only one functional allele of *Apc* with subsequent loss of the wild type allele leading to aberrant Wnt signalling[72] and the *Muc2*$^{-/-}$ mouse which develop spontaneous intestinal and colonic adenomas in response to chronic inflammation[73]. A 12-week feeding programme of 10% freeze-dried BRB diet significantly decreased tumour incidence and tumour multiplicity in *Apc*1638$^{+/-}$ mice by 45% and 60% respectively. Similarly, in *Muc2*$^{-/-}$ mice, feeding of BRBs reduced tumour incidence and multiplicity by 50%. BRBs appeared to inhibit proliferation in both models, whereas terminal differentiation of goblet cells was not altered, potentially due to a relatively short feeding programme. Quantification of other cell types, such as enteroendocrine, Paneth and enterocytes may provide a better insight into the effects of BRBs on intestinal cell differentiation in the future. Mechanistically it was reported that reductions in tumourigenesis within the two models was via two different biological pathways. BRBs elicited anti-tumour effects in *Apc*1638$^{+/-}$ mice through inhibition of Wnt/β-catenin signalling and its downstream targets, including *c-Myc* and *CyclinD1*, whereas in the *Muc2*$^{-/-}$ mouse BRBs down-regulated cytokines commonly associated with inflammation, including Cox-2, Tnf-α, IL-1, IL-6 and IL-

10[71]. These models were the first to suggest potential mechanisms of action of BRBs against *in vivo* CRC development.

The hereditary condition FAP is characterised by an early onset of colonic polyposis which confers an increased risk of developing CRC. A key phase Ib study was conducted to investigate the effects of BRBs on FAP patient rectal polyps[74]. Patients with at least 5, ≥2mm rectal polyps at baseline endoscopy were enrolled into the study and randomly divided into two groups consisting of 7 patients each. Group 1 received placebo powder slurry (maltodextrin and dextrose) taken orally 3 times a day (60 g/ day in total) and two BRB rectal suppositories at night (each containing 720 mg BRB powder). Group 2 received a BRB powder slurry 3 times a day (60 g/ day in total) plus two BRB rectal suppositories. BRB intervention was continued for up to 9 months. The study showed that, despite 3 non-responders, BRB rectal suppositories were sufficient to reduce polyp burden and regress rectal polyps, but there was no added benefit from oral BRB powder consumption[74]. Within responding adenomas, BRB treatment decreased cellular proliferation, and positively impacted on epigenetic marks and proteins by decreasing promoter methylation, but had no effect on cell death[74]. These results suggest that BRBs may be a potential adjuvant treatment to improve the outcome for FAP patients.

1.8.3.1 Black raspberries and epigenetics

Epigenetics refers to the study of the heritable and dynamic chemical modification of a cell's transcriptional capacity, without any alteration to the genetic sequence[75]. Epigenetic mechanisms include but are not limited to histone modification, modulation of DNA methylation status and miRNA-mediated manipulation of translation; these mechanisms regulate gene expression and can impact upon tumourigenesis. Several major enzymes that catalyse these mechanisms: DNA methyltransferases (DNMTs); ten eleven translocation (TET) enzymes; histone acetyltransferases (HATs) and histone deacetylases (HDACs)[75]. Metabolites resulting from processed dietary phytochemicals and fibre can affect these enzymes and epigenetic mechanisms, and ultimately gene expression. Specifically, in the context of cancer, genes involved in tumour suppression are usually hypermethylated resulting in epigenetic gene silencing; a mechanism that supports tumour survival[76].

Previous studies by Wang *et al.* have reported an association between BRB-derived anthocyanins and a decreased hypermethylation status seen in CRC, suggesting that BRBs could play a role in modulating epigenetic events[77]. Typically, promoter regions of Wnt pathway antagonists such as *SFRP2*, are hypermethylated in human CRC, leading to aberrant Wnt signalling and tumour progression. Human CRC cell lines treated with BRB-derived anthocyanins for three days at 0.5, 5, and 25 µg/ml suppressed DNMT1 (maintenance DNA methyl transferase) and DNMT3b (*de novo* DNA

methyl transferase) activity. Additionally AC exposure resulted in the reactivation of Wnt pathway repressors, CDKN2A, SFRP2, SFRP55 and WIF1, via promoter demethylation. This resulted in the induction of apoptosis, cell cycle arrest and mRNA suppression of key Wnt regulators, β-catenin and C-MYC (a downstream target of the Wnt pathway), in these CRC cell lines[78].

The role of BRBs on epigenetic silencing has been evaluated in precancerous murine models that recapitulate human ulcerative colitis (UC, an intermediary step of colon carcinogenesis that is also influenced by deregulation of the Wnt pathway). IL-10 knockout UC mice fed a 5% BRB diet had less colonic ulceration as a result of reduced nuclear localisation of β-catenin than respective control mice[79]. This study also reported that mRNA expression of Wnt repressors was significantly upregulated in BRB treated mice, which coincided with hypomethylation of the same gene promoters and decreased protein levels of methylation-regulating proteins DNMT, HDACs and methyl-binging domain proteins (Mbd2), compared to control fed UC mice[79]. Studies from the same group using a dextran sodium sulfate (DSS)-induced, an irritant that induces extensive colonic mucosal inflammation, UC model reported that 5% BRB diet significantly reduced colonic ulceration and decreased protein levels of Dnmt3B, Hdac1, Hdac2 and Mbd2 and reduced promoter methylation of Wnt repressor genes[80]. In AOM(azoxymethane) /DSS chemically induced murine cancer models, diets containing 3.5 µmol/g and 7 µmol/g BRB-derived ACs, which corresponded to the AC concentrations in 5 and 10% freeze-dried BRB powder respectively, resulted in significant reduction in Dnmt1 protein expression. However, BRB anthocyanins had no effect on TET enzyme mRNA expression (which mediate active DNA demethylation) in the intestinal epithelium at either concentration[81], this suggests that BRB-induced demethylation is a passive process in this context. Similarly, to other studies, the cancer preventative effect of BRBs was also linked to the promoter demethylation of *Sfrp2*, and subsequent increase in Sfrp2 protein concentration negatively regulating Wnt/β-catenin signalling[81].

In a phase 1 clinical study of 20 CRC patients who were treated with oral BRB powder (60 g/day) for 1-9 weeks, epigenetic changes were only observed in patients who only received on average 4 weeks of BRB intervention, with reduced promoter methylation of key Wnt pathway inhibitors and decreased expression of DNMT1[77]. Epigenetic changes were confirmed in a subsequent phase 1b clinical trial, in which 14 FAP patients received BRB intervention through rectal suppositories with or without the addition of oral supplementation for 9 months. Here the majority of patients (11/14) responded to the BRB treatment with polyp regression and significant decreases in protein levels of DNMT1 with reactivation of the silenced p16 promoter[74]. Providing direct clinical evidence for berry mediated chemoprevention.

Aside from BRB anthocyanins, the dietary fibre obtained from sources such as BRBs can also protect against tumourigenesis through several mechanisms. Firstly, increased speed of colonic transit helps minimise epithelial exposure to carcinogens and secondly, metabolism of fibre by gut bacteria, such as *Anaerostipes* or *Clostridia*, into short-chain fatty acids (SCFAs) such as butyrate, acetate and propionate, can cause tumour-influencing epigenetic alterations[82]. SCFAs are a source of energy but also act as signalling molecules within the body. Butyrate is the main source of energy for colonocytes and can also induce apoptosis in cancer cells[83]. Intracellular butyrate and propionate can inhibit gut HDAC activity, promoting hyperacetylation of histones (limiting methylation and thus reducing epigenetic silencing of anti-tumourigenic genes) and can impact on signal transduction[84]. H3 acetylation in the promoter and conserved non-coding (CNS1) regions of the Foxp3 locus (which controls colonic T-regulatory cell population thought to be cancer preventative) is enhanced by butyrate-mediated HDAC inhibition[85]. Butyrate, along with propionate and acetate, activate free fatty acid receptor 2 (FFAR2), the receptor for short-chain fatty acids which regulates whole-body energy homeostasis and is an important regulator of inflammation involved in enhancing T-reg cell accumulation. Stimulation of FFAR2 via SCFAs is essential for innate immunity and chemoattractant-induced responses; SCFA-mediated T-reg accumulation is important in limiting inflammatory responses associated with tumourigenesis[85, 86]. In disease states or conditions resulting from inadequate diets, such as CRC, the levels of SCFA may be diminished, which can mitigate the protective effects of SCFA-mediated immune regulation[87].

In more recent years there have been emerging roles of microRNAs (miRNAs, small noncoding RNAs) in epigenetic regulation. miRNAs can act as epigenetic regulators post-transcriptionally, altering target mRNAs and interfering with protein translation without modifying gene sequences. This is often via the induction of mRNA degradation or repression, physical interaction of miRNAs with RNA interference machinery[88]. In addition to being epigenetic regulators, miRNAs can themselves be regulated by the epigenetic machinery involved in DNA methylation and RNA and histone modifications. miRNAs have been implicated in several fundamental biological processes such as proliferation and migration. The impact of miRNAs on physiological and pathophysiological processes can be either beneficial or detrimental depending on the specific miRNA and its target, and dysregulation of this complex miRNA-epigenetic feedback loop can contribute to tumourigenesis[88]. Studies have shown that diet, and the subsequent downstream effects of dietary components on the microbiome and production of metabolites can influence and interact with miRNAs. miR-92a, a member of the miR-17-92a cluster important in regulating cell cycle, proliferation and apoptosis has been shown to be 7x higher in human CRC tissue compared to adjacent healthy tissue from the same patient[89]. miR-92a has been implicated in tumour development through its suppressive interaction

with the anti-apoptotic molecule, BCL-2-interacting mediator of cell death (BIM), in human CRC cell lines and colonic cancer tissue[90]. In human CRC cell lines, microbe-derived butyrate has been shown to reduce the levels of miR-92a and other miRNAs belonging to the miR-17-92a cluster. A cancer protective role of butyrate was identified via butyrate's silencing effect on c-Myc protein levels leading to a reduction in c-Myc-induced miR-17-92a promoter activity and ultimately reducing levels of oncogenic miR-92a synthesis[89]. A recent study has identified a BRB-mediated chemopreventative association with miR-24-1-5p in CRC through inhibition of Wnt/B-catenin signalling and subsequent downstream targets involved in CRC tumourigenesis[91]. Utilising the colitis-induced CRC model (AOM/DSS), expression levels of miR-24-1-5p was found to be low in tissues from AOM/DSS-induced mice but was significantly increased in AOM/DSS mice following 9-week feeding intervention with BRB anthocyanins (7µmol/g BRB anthocyanins equating to a 10% BRB diet)[92]. It was shown that upregulation of miR-24-1-5p in human CRC cell lines leads to inhibition of cancer cell proliferation, migration and survival, by suppressing β-catenin protein levels and modulating downstream target gene expression[92]. FAP patients who responded to 9 month BRB suppository intervention had 37 unique miRNAs that were demethylated in adenomas, whilst in these same patients promoter methylation of Wnt suppressors was not altered, indicating the uniquely demethylated miRNAs regulate the Wnt signalling pathway[74]. Together these studies highlight a potential mechanism whereby BRBs and their anthocyanin derivatives can mediate tumour suppression through miRNA epigenetic interaction with β-catenin and downstream Wnt target genes. We are yet to fully establish the chemopreventative relationship between BRBs and epigenetic gene regulation in the colon, however, there is increasing evidence suggesting that BRBs and their microbial-derived metabolites play roles in regulating the epigenome and can influence carcinogenesis.

1.8.3.2 Black raspberries, microbiome and the metabolome

The microbiota of the human gut is a symbiotic web consisting of trillions of microorganisms: primarily bacteria, but also viruses, archaea, fungi and protozoa[93]. The human gut microbiome refers to the collective genomes of these microorganisms, in which there are approximately 3.3 million genes encoded[94]. The importance of the gut microbiota on human physiology has become increasingly apparent over the past decade. With the advent of 'omic' technologies and the rapidly developing analytical and sequencing techniques that follow we can now study the impact of the microbiota on health and disease in great detail.

The human microbiota is often referred to as a hidden organ, with its own physiology and pathophysiology[95]. It is essential in regulating normal gut homeostasis and health and is a relatively stable entity once it has been established. However, the microbiome can be manipulated and altered through intervention with antibiotics or changes in diet, resulting in dysbiosis and disease. Dysbiosis

of the intestinal microbiota, contributes to many disease states including obesity, diabetes and inflammatory bowel disease, all of which may predispose to CRC[96, 97].

Excessive consumption of dietary elements such as protein, sugar and saturated fatty acids can adversely affect the gut microbiota, decreasing microbial diversity and resulting in inflammation and decreased production of butyrate[98]. Intake of fibre and polyphenols positively influence the microbiota, resulting in increased frequency of beneficial gut microbes that exert biological effects including reduced inflammation, increased antioxidant production and increased butyrate production[99]. BRBs, and the abundance of fibre and polyphenols such as anthocyanin and ellagitannin contained within them, are capable of altering the composition and metabolic potential of the gut microbiota[100]. A BRB diet has been shown to positively influence the ratio of beneficial gut microbes including those of the genera *Anaerostipes, Akkermansia, Desulfovibrio* and members of the phylum *Bacteroidetes*[99, 101].

Metabolomic studies have identified an enrichment in several beneficial metabolic pathways and metabolites, including butyrate, in mice fed a diet rich in BRB[98]. Rats fed a BRB diet showed an increase in butyrate-producing bacteria such as *Anaerostipes* when compared to a control diet[99]. It has been shown that FFAR2, the receptor for short-chain fatty acids, is required for the beneficial effects of BRBs; indicating bacterial fermentation is an important mechanism underpinning BRB-mediated benefits[102]. Colonic inflammation and the subsequent cytokine and ROS generation results in an increase in opportunistic, adhesion-invasive bacterial species such as *Escherichia coli* that further promote inflammation, and a decrease in species from *Akkermansia* and *Desulfovibrio* genera that have anti-inflammatory effects[101]. Intervention with a BRB diet can reduce inflammation and the increased risk of tumourigenesis associated with it by increasing the abundance of these anti-inflammatory bacterial species and via increased activity of FFAR2, which has been implicated in suppressing colonic inflammation.

Several studies have demonstrated the association of an increased *Firmicutes* to *Bacteroidetes* ratio with diet-induced obesity[103]. *Firmicutes* are positively selected for in obesity as member species have an increased capacity for processing polysaccharides, a major component of diet-induced obesity[104]. Furthermore, it has been reported that obese individuals have a decreased population of *Akkermansia muciniphila* which, as previously stated, is a bacteria that has anti-inflammatory effects as well as anti-diabetic and anti-obesity functions, including roles in glucose homeostasis and adipose metabolism[101]. A BRB rich diet has been shown to modulate the murine gut microbiota, increasing the abundance of *Bacteroidetes* compared to *Firmicutes* and the abundance of *A. muciniphila*[101]. This microbial modulation via BRB intervention provides a number of bacterial metabolic alterations, including increased biosynthesis of vitamins and essential amino acids, that

provide health benefits to the host such as reduced inflammation and increased cardiac and neural tube protection[101, 105]. Regulating inflammation and helping to maintain a healthy weight are some of the key elements of BRB-mediated chemoprevention.

The phytochemicals contained within BRBs are processed and absorbed by the various microbes in the gut but the subsequent metabolites can be transported through the body and exert systemic beneficial effects. Metabolomic approaches have shown alteration in over one hundred metabolites across the colonic mucosa, liver and the faeces of healthy mice fed a BRB supplemented diet. BRB supplementation decreased certain lipid metabolites in the colon including long-chain fatty acids and omega-6 polyunsaturated fatty acids, which can contribute to insulin resistance, inflammation and obesity when consumed in excess. Conversely, BRBs led to an increase in cancer protective omega-3 polyunsaturated fatty acids (ω-3 PUFA), such as linolenate, in the liver[106, 107]. Linolenate is converted to docosapentaenoate (DPA), docosahexaenoate (DHA) and eicosapentaenoate (EPA), each of which has been shown to provide metabolic, anti-inflammatory and neuroprotective benefits[108]. Clinical studies have also indicated an improvement in body weight and physical, cognitive and social functions in lung cancer patients given a daily supplement of DHA and EPA, further demonstrating how BRBs exert their chemoprotective effects[109].

Several species of bacteria that have generated interest due to their tumourigenic potential in the gastrointestinal tract, namely: *Fusobacterium nucleatum, Escherichia coli and Bacteroides Fragilis.* In both cell lines and patient-derived xenograft (PDX) murine models, the presence of *F. nucleatum* increases tumour cell proliferation and overall tumour growth rates[110]. *E. coli* that express the genomic island polyketide synthase can produce DNA alkylating genotoxins that may result in mutagenic DNA damage leading to tumourigenesis[111]. *B. Fragilis,* along with *F. nucleatum* and *E. coli* can create a tumourigenic biofilm that facilitates bacterial epithelial infiltration, recruits immune cells, and promotes inflammation in precancerous colonic lesions[112]. BRB intervention has been shown to increase the abundance of butyrate-producing bacteria; butyrate stimulates mucus production and maintains epithelial integrity, possibly protecting against the adverse effects resulting from biofilm generation[113].

As previously described, the microbiota has the capacity to influence many disease states in the gut. Pathobiotic bacteria, such as *E. coli,* which accumulate in a poor-diet or disease-state gut, can penetrate the gut epithelium and generate toxins that promote colonic inflammation and tumourigenesis[114]. Studies using mice have shown that in circumstances such as these, antibiotics can be used to suppress this pathobiotic accumulation, and ultimately, the adverse effects associated with it[115]. Conversely, the use of antibiotics can also reduce the frequency of beneficial gut microbes and depletion of the gut microbiota may encourage the growth of antibiotic-resistant bacteria that

can further induce inflammation[116]. The gut microbiota is essential in facilitating the beneficial effects of BRBs and any perturbation of the gut microbial content has the capacity to interfere with these effects. Administration of a 5% BRB diet to *Apc*$^{Min/+}$ mice (which mimics FAP disease) did not effect polyp development in mice that had been treated with antibiotics (1 g/L ampicillin, 1 g/L neomycin, 1g/L metronidazole, and 0.5 g/L vancomycin in the drinking water)[102]. This demonstrates that the gut microbiota is indeed required for BRB-mediated chemoprevention. Homozygous deletion of *Apc* within the ISC compartment of mice, that recapitulates the initial stages of CRC initiation, has also been shown to alter the composition of the microbiota as seen with trends of increasing abundance of *Bacteroides* and *Clostridium XVII*[117] which can contribute to the tumourigenic biofilm. The gut microbiota is affected very early on in tumourigenesis and has the potential to serve as an early diagnostic tool. Mice fed a diet of 10% BRBs from 2-weeks prior to induction of ISC *Apc* loss have a reduced impact to the gut microbiota upon tumourigenesis, indicating that BRBs can abrogate the initial cancer-mediated alterations to the microbiota and promote a favourable *Bacteroidetes* to *Firmicutes* ratio which supports a leaner bodyweight[117]. Further work with larger sample sizes is needed to fully characterise the effect of BRBs on the composition and function of the microbiota in CRC.

1.8.3.3 Black raspberries, inflammation and immunity

Chronic inflammation is a major risk factor for CRC and is considered a hallmark of cancer[4]. Inflammation involves a complex network consisting of numerous immune and inflammatory cells, cytokines, chemokines and pro-inflammatory mediators[118]. Inflammation and subsequent accumulation of immune cells in inflammatory areas can lead to the activation of signalling pathways that promote tumour growth and invasion. Increased activation of inflammatory signalling pathways can often lead to genetic instability, an increased incidence of oncogenic mutations and result in inflammation-mediated carcinogenesis[119]. BRB intervention can help to reduce inflammatory-mediated tumourigenesis through its inhibition of certain pro-inflammatory mediators and its regulation of immuno-modulatory cells[23, 24].

The pro-inflammatory mediator COX-2 is elevated in many cancers including CRC and can be rapidly and transiently induced by inflammatory cytokines and growth factors[120]. COX-2 is an enzyme responsible for the production of prostaglandins that enhance tumour growth and can aid in tumour evasion from the immune system by modulating the tumour microenvironment[121]. A further role of COX-2 in tumourigenesis is its capacity to increase tumour invasiveness and metastatic potential[122]. Several studies have demonstrated that BRB diet can suppress COX-2 expression through inhibition of the transcription factor, nuclear factor-κB (NF-κB), which is involved in signalling pathways (e.g. MAPK signalling), proliferation, cell adhesion and immune/inflammatory

modulation[123-125]. In murine macrophages stimulated by the endotoxin lipopolysaccharide (LPS), administration of an anthocyanin-rich BRB fraction reduced LPS-induced inflammation (by dampening iNOS expression) and through inhibition of the MAPK pathway[126]. More recently a unique phytochemical from berry fruits known as triterpenoid glycosides have been implicated in disease prevention. Specifically, oral administration of a triterpenoid-rich fraction (TFRC, 25, 50, or 100 mg/kg/day) from the Korean black raspberry (*Rubus coreanus*) has been shown to ameliorate colitis in DSS-treated mice, maintaining crypt structures and reducing markers of inflammation including reductions in *Tnf-α, IL-1β*, and *IL-6* mRNA levels. TFRC protected against colitis and in turn carcinogenesis by reducing macrophage accumulation in mice and inhibited nitric oxide and prostaglandin production by suppressing iNOS and Cox-2 levels, downstream inflammatory cytokines and members of the MAPK/NF-κB pathway *in vitro*[127]. Similarly, in DSS-induced colitis mice, a 5% BRB diet reduced macrophage and neutrophil accumulation and suppressed nuclear translocation of NF-κB[80].

Natural killer (NK) cells are a type of innate immune lymphocyte that exhibits cytotoxic activity, mediating anti-tumour and anti-viral responses[128]. Depletion of NK cells substantially encourages CRC development, whereas increased NK cell tumour infiltration is associated with a better prognosis, demonstrating the importance of these cells in mitigating tumour progression[129]. In two colitis-associated CRC mouse models, AOM/DSS and $Apc^{Min/+}$/DSS which have a depletion of NK cells in adenomas, a diet supplemented with 5% BRB powder suppressed colon polyp size and frequency whilst enhancing NK cell tumour infiltration[130]. Moreover, in colon biopsies from CRC patients treated with oral BRB slurry, BRBs were shown to increase NK cell tumour infiltration[130]. In addition to NK lymphocytes, another immune cell population that has important immune surveillance roles are T cell lymphocytes. Cytotoxic killer, CD8+, T cells are involved in immune-mediated cell death and can also act as cytokines, CD4+ T cells, known as helper T cells, secrete cytokines to assist with the immune response, and regulatory T cells mainly act to suppress T cell-mediated immune response. In cancer T cells often become dysfunctional usually aiding cancer progression[131]. BRB-ethanol extract and two abundant BRB phytochemical metabolites (Cyanidin-3-Rutinoside and Quercitin-3-Rutinoside) can modulate the proliferative capacity of the human CD4+ and CD8+ T lymphocytes. All compounds were able to inhibit activated-T cell proliferation and survival and reduced differentiation of myeloid-derived suppressor cells (MDSC, cells capable of priming the metastatic niche) *in vitro,* through modulation of the JAK/STAT pathway which has documented roles in MDSC differentiation. Prior studies have shown that the BRB phytochemicals, Cyanidin-3-glucoside anthocyanin, Quercetin and Rutin can inhibit JAK/STAT and MAPK signalling[132, 133]. The beneficial effects of BRBs on inflammation are diverse and numerous, *in vitro,* the anthocyanin component of

BRBs has been more effective at suppressing inflammation than the commonly used anti-inflammatory drug 5-aminosalicylic acid[134], demonstrating how BRB and their metabolites can be an effective and accessible drug option for treatment of inflammation and inflammation-associated cancer.

2. Conclusion

The potential of BRBs as a cancer therapeutic is indisputable, as daily administration of BRBs have shown to reduce colorectal tumour/ polyp numbers, oral SCCs and severity in humans with minimal adverse effects[45, 74, 77]. Extensive research has revealed that BRBs have pleiotropic effects against all stages of carcinogenesis by promoting apoptosis and differentiation, reducing proliferation, modulating the epigenome, the microbiome and the metabolome, reducing tumour-mediated inflammation and oxidative stress and inhibiting angiogenesis (Figure 3). Collectively, these studies support the potential of BRBs for therapeutic cancer intervention and highlight the ability of BRBs to limit the window of opportunity of cancer initiation and progression. However, they highlight the complexity of the involvement of endogenous factors in carcinogenesis and emphasises the importance of understanding the precise mechanism in which dietary and drug interventions protect against cancer. Moreover, translation into the clinic has not been straightforward, with varied responses occurring among patients[74, 77]. This is likely to reflect inter- and intra-individual and tumoural heterogeneity, differences in molecular signalling and enzyme activity within tumours and variances in individuals' microbiota and metabolism. In addition, the limited bioavailability of anthocyanins, the concentration, frequency, duration, delivery method and delivery matrix of BRB interventions has differed across studies, which could also be a confounding factor for reporting beneficial outcomes. BRBs have been administered as a slurry of freeze-dried powder suspended in water, as rectal suppositories, as topical agents delivered in a bioadhesive gel, or as dissolvable slow-release BRB troches. It is important to note that the concept of chemoprevention is not to radicalise our lifestyle, but to identify ways in which to adopt a healthier lifestyle and reduce the window of opportunity of cancer development by lessening the risks. Patient compliance is often a rate limiting step in the success of chemopreventative strategies, and so it is important that such strategies are easy to adhere to. While it remains difficult to accurately estimate the concentration of BRB consumption for individual patients to have beneficial effects, it is necessary that the regime is simple and does not require the consumption of hundreds of berries a day which would not be a sustainable lifestyle change. It is also likely that for each given cancer type and predisposing disease that a specific BRB dose, delivery method and regime is utilised to maximise the efficacy of the treatment.

It is also important to note that the chemopreventative effects of BRBs are not attributed to a single BRB compound but collectively from anthocyanins, ellagic acid, flavonoids, fibre, other

antioxidants and phytochemicals such as quercetin and rutin, and BRB metabolites which may individually impact on specific cellular processes, or work collectively to dampen down signalling pathways and prevent feedback loops and cross-talk among pathways that are fundamental for tumour development. Additionally, edible berries have proven to protect against cancer before the transformation of a premalignant cell, by aiding in the maintenance of a healthy body weight and supporting the growth and abundance of beneficial gut microbes that protect against diseases which predispose individuals to cancer such as obesity, diabetes, gastroesophageal reflux and inflammatory bowel diseases.

The best documented anti-cancer effects of BRBs have been identified in oral, oesophageal and colorectal cancers, it is clear that cancer can act systemically affecting the function of other organs and resulting in metastasis. While research has started to focus on investigating the chemopreventative roles of BRBS in breast[135] and prostate cancer[136], future studies should focus on the impact of BRBs and their metabolites on 1) the systemic implications of cancer, 2) the ability of polyphenols to prevent metastasis at secondary sites, 3) the impact of BRBs on liver-intestinal cross talk due to the roles of the liver in metabolism and ultimately 4) the impact of BRB polyphenols on cancer stem cell or stem-cell like populations.

3. Figure Captions

Figure 1: Endogenous and Exogenous Risk Factors of Cancer Development

Cancer arises from abnormal and uncontrolled cell division. Cancer progression is the result of a complex interaction between innate host factors (endogenous risk factors), such as inherited mutations and epigenetic signatures, disturbances in microbial diversity due to chronic inflammatory diseases and reduce immune function, with exogenous factors that can increase mutational risk over time such as UV exposure, smoking, physical inactivity, obesity, inadequate dietary intake and alcohol consumption.

Figure 2: Overview of Black Raspberry Metabolism

Schematic representation of the organs involved in the absorption and metabolism of black raspberry polyphenols.

Figure 3: Summary of the Cellular Processes Involved in BRB-Mediated CRC Chemoprevention

Black raspberries exert chemopreventative and therapeutic effects against colorectal cancer by acting on several cellular functions, biological processes and signalling pathways that are all interlinked. The protective effects of black raspberry arises from a complex interaction and cross-talk of BRB polyphenols on genes and proteins associated with epigenetics, inflammation, oxidative stress,

proliferation, cell cycle regulation, apoptosis, angiogenesis, metastasis and via modulation of the gut microbiome. So far little is known about the involvement of the intestinal stem cell compartment in BRB-mediated cancer prevention. ⍰ = Inhibition; ↓ = Decrease, ↑= Increase

4. Competing Interests

The authors declare no conflict of interest.

5. Contributors

SM was involved with the literature search, figure design, and drafting and critical revision of the manuscript. CP was involved with the literature search and drafting of the manuscript. LP was involved in drafting and critical revision of the manuscript.

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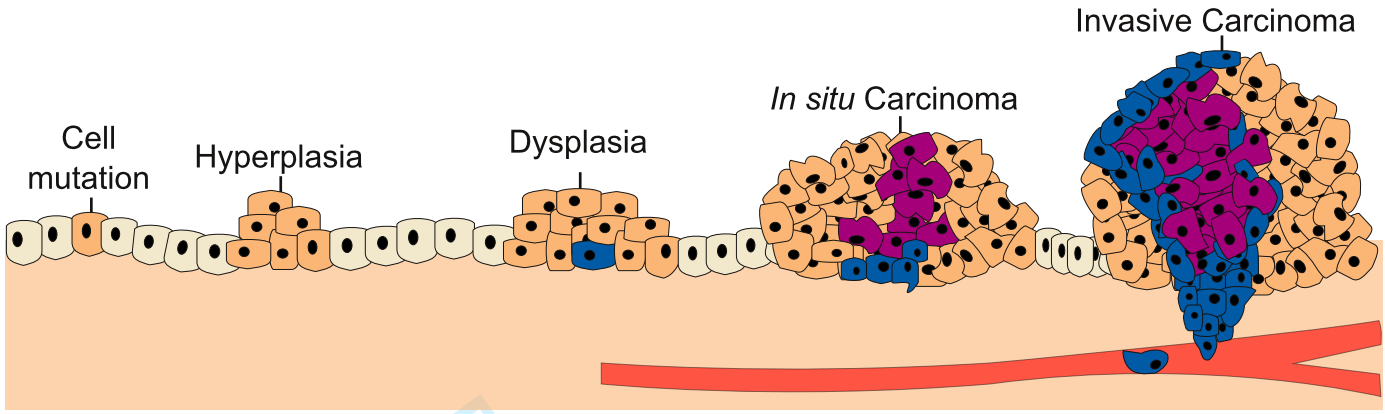


CANCER PROGRESSION

Genetics, Eigenetics, Age, Gender, Metabolic state, Inflammation, Immune function, Hormones and Signalling Pathways, Microbiome, etc.

Endogenous Factors

Host Factors

Environmental Factors

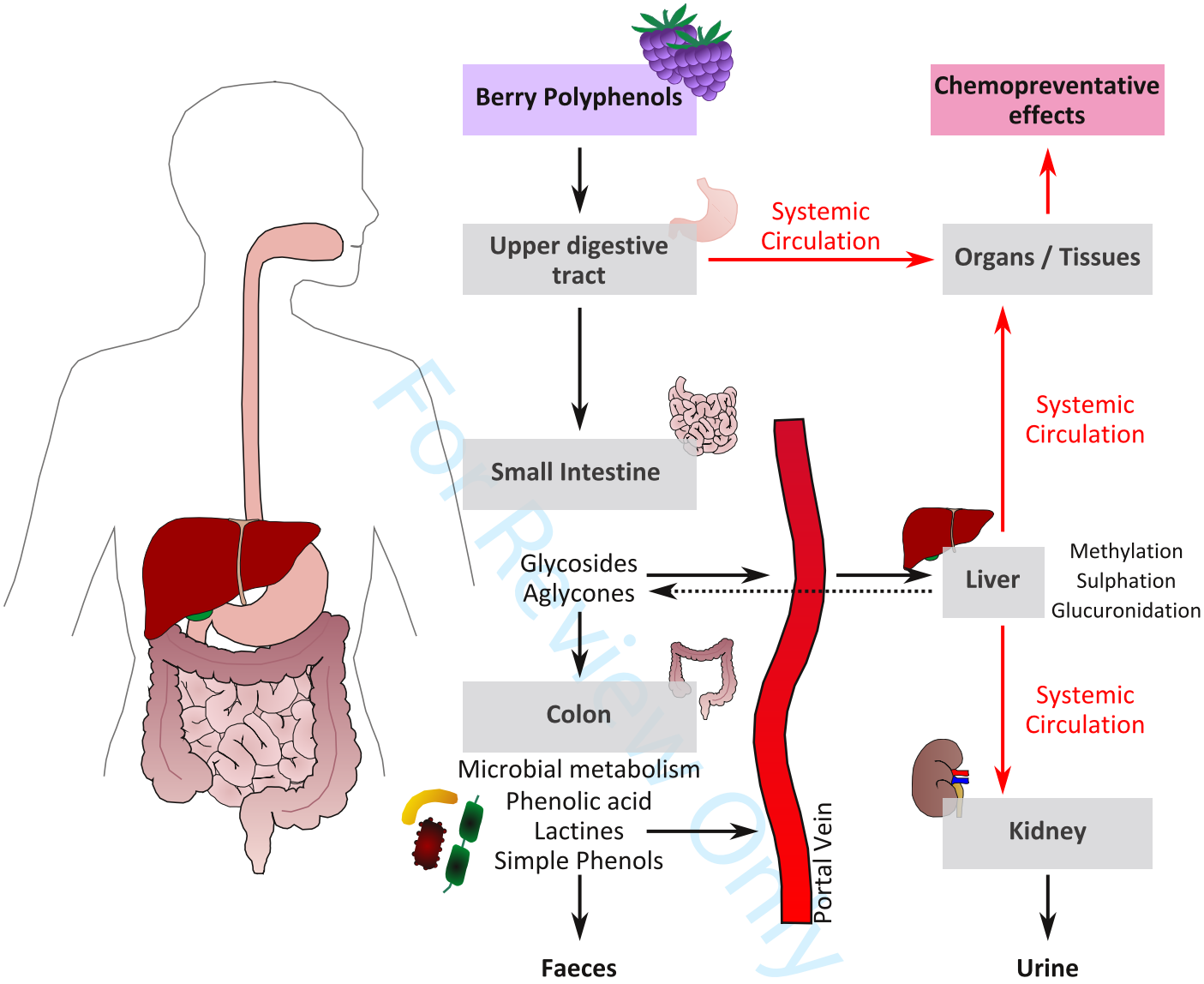
Lifestyle Factors

Infectious agents, Solar and other radiation, Food contaminants, Environmental carcinogens

Exogenous Factors

Nutrients, Energy Intake, Diet, Alcohol, Physical Activity, Smoking, Obesity, Other lifestyle factors

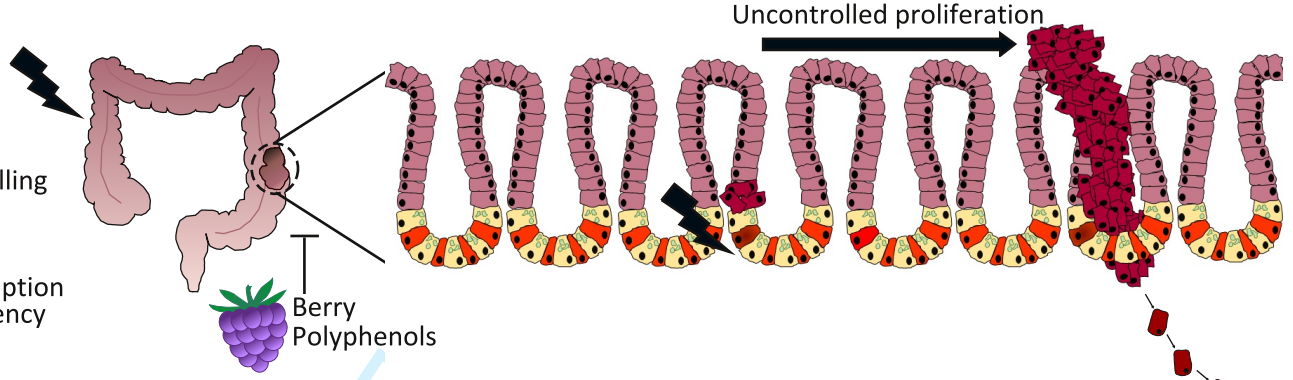
Exogenous Factors



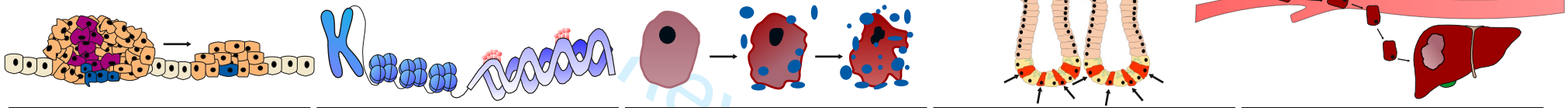
Chemopreventative effects of black raspberry polyphenols on CRC

Endogenous Factors
Genetic mutation
Epigenetics
Age and gender
Inflammation
Immune function
Metabolic state
Microbial dysbiosis
Hormones and signalling pathways

Exogenous Factors
High alcohol consumption
Diet/ nutrient deficiency
Excess bodyweight
Physical inactivity
Smoking



Inhibit Angiogenesis
↓ VEGF
↓ C-MYC
↓c-jun
↓c-fos



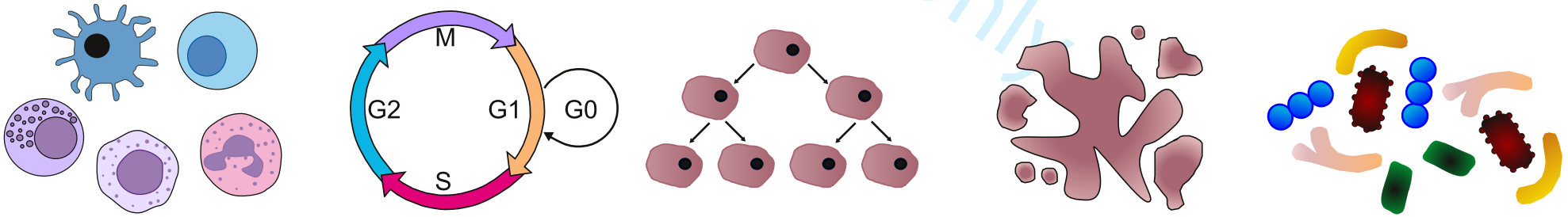
Reduce Tumour Burden
↓Dysplastic lesions/ tumour incidence
↓Tumour mulitplicity
↓FAP polyp size

Epigenetic Regulation
↓DNMT and HDAC expression and activity
↓ Tumour supressor gene promoter methylation
Regulates miRNA expression

↓Oxidative Stress
↓ Reactive Oxygen Species
↓ Reactive Nitrogen Species
↓ iNOS
↓ DNA adduct formation

Intestinal Stem Cells
?

Inhibiting Metastasis
↓COX-2 tumour invasiveness and metastatic potential
↓MDSC differentiation which are capable of priming the metastatic niche



Dampen Inflammation
↑ NK cell tumour infiltration
↓ Immune cell trafficking
↓ NF-KB nuclear translocation
↓ COX-2, IL1β, IL6, IL10, TNF-α
↓ iNOS
↓ JAK/STAT signalling
Inhibit activated-T cell proliferation

Cell Cycle Regulation
↑p21, p16, p27
↓Cyclin-dependent kinases
↓CyclinD1, CyclinB1, CyclinA

Reduce Proliferation
↓ Ki67, BrdU, PCNA
↓ β-catenin protein levels
Inhibit signalling pathways:
Wnt/ β-catenin pwathway
MAPK/ERK pathway
PI3K/ AKT/ mTOR

Promote Apoptosis
↑Caspases-3, -8 and -9
↑p53
↑BAX
↓Bcl-2/ BIM

Maintains Microbial Diversity
↑*Bacteriodes* (also maintains bodyweight)
↓ *Firmicutes*
↑Butryate levels (pro-apoptotic, modulates epigenome, supresses c-Myc)
↓ Inflammation