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An immunological perspective for preventing cancer with berries

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Abstract. Berries and their phytochemicals have well documented chemopreventive roles, but understanding their ability to regulate cancer immunology is only beginning to be explored. The literature, including human studies, suggests that berry components can modulate our immune system to delay cancer development. Moreover, their wide spectrum of phytochemicals suggests that they might influence the functions of multiple immune cells and different aspects of cancer immunity. Cancer immune-therapies are showing promise for some types of cancer because they boost T cells' ability to recognize tumor cells – an essential prelude to destruction. Recognition occurs after dendritic cells present antigen, such as tumor antigen, to T cells, generating an adaptive response. Therefore, the potential of berries to aid cancer immune-therapies by, for example, regulating dendritic cells, warrants further investigation in animal and human studies. More information is also needed about berries' effects on the entire spectrum of immunity so that a comprehensive view can inform efforts to use berries to enhance immune responses during cancer prevention and treatment. This review summarizes the effects of berries as anti-tumor agents from the immunological perspective in tumor-bearing animals and humans.

Keywords: Cancer immunology, berries, natural killer cells, T cells, dendritic cells

1. Introduction

Berries contain abundant phytochemicals that have been shown to delay cancer development through multiple mechanisms such as altering gut microbiome and host metabolome, but understanding their ability to regulate cancer immunity is only beginning to be explored [1–10]. It is known, however, that phytochemicals can modulate the immune response by targeting key immune cells that control the pro- or anti-inflammatory microenvironment, thus helping to suppress tumor progression [11]. Moreover, these compounds vary so greatly in structure and function that they can target a wide range of cancer cells, immune cells and their cytokines [12–26].

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Berry is a simple indehiscent fruit (it does not split open to release its seeds when ripe), has a few or many seeds, and is derived from a single, simple, or compound ovary [27]. Thus, berries include many commonly consumed fruits and vegetables, such as strawberries, blueberries, blackberries, red raspberries, black raspberries, cranberries, grape, kiwi, banana, tomatoes, eggplant, cucumber, watermelon, etc., as well as many uncommon types, such as gooseberries, Goji berries, elderberries, noni *(Morinda citrifolia*), and acai (*Euterpe oleracea* Mart.), etc. We used these terms and "cancer immunity" to search PubMed, and mainly focused on animal cancer models and human studies. Therefore, this review summarized the findings of berries as anti-tumor agents in an immunological perspective view.

2. Cancer immunology

Innate and adaptive immunity are the two immune responses. Innate immunity involves dendritic cells (DCs), macrophages, neutrophils, natural killer (NK) cells, granulocytes, basophils, eosinophils, and mast cells. Adaptive immunity involves predominantly T cells and B cells [1]. Immune defense mechanisms also employ various soluble factors, such as chemokines, cytokines, and immunoglobulins. Both innate and adaptive immunity closely interact with each other. For example, antigen-presenting cells (such as DCs and macrophages) identify and "present" cancer cells to effector cells (such as T cells and B cells), which then destroy them. Recently, cancer immune-therapies have generated intense interest [28]. DCs are an important target for generating specific anti-tumor immunity [29], as they trigger adaptive responses by presenting tumor antigens to T cells. T cells are categorized by cell membrane markers such as CD4 and CD8 [1]. $CD8^+$ T cells, which secrete interferon gamma (IFN-γ), have cytolytic activity against tumor cells [30]. Interestingly, this cytolytic activity and the persistence of $CD8^+$ T cells depend largely on the action of $CD4^+$ T helper cells [30]. Thus, one key to an optimal response against cancer is to establish a tumor-specific $CD4^+$ T helper cell response [30].

NK cells spontaneously kill cells that are deemed to be dangerous to the host, such as cancer cells, and thus are presumed to be key effectors in cancer immune-surveillance [31]. NK cells are usually defined as $CD3^-CD56^+$ in humans and $CD3^-NK1.1^+$ or $CD3^-NKp46^+$ in mice [31]. In humans, these cells account for 5%–15% of circulating lymphocytes in the blood. Several mechanisms enable NK cells to distinguish healthy cells from target cells. These mechanisms integrate signals from different receptors and form the basis of NK cell activation [31]. NK cells secret IFN-γ and they express inhibitory receptors of the major histocompatibility complex (MHC) class I [31]. Binding of self MHC class I to the developing NK cells allow their "licensing" and the tolerance of NK cells [31]. However, cells undergoing malignant transformation often lose their expression of MHC class I molecules, thereby escaping NK cells' surveillance [31].

The complexity of the tumor microenvironment determines the outcome of immune cells, especially those with dual functions, such as macrophages and neutrophils [32]. Tumor-associated macrophages derived from circulating monocytes are among the most abundant cells in the tumor microenvironment [33, 34]. Normally, they promote both innate and adaptive immunity and phagocytize dead or dying cells and cell debris [35]. In the tumor microenvironment, however, tumors re-educate macrophages to promote tumor growth and spread [34]. Thus, tumor-associated macrophages suppress adaptive immunity and enhance angiogenesis, tumor cell invasion, and intravasation into blood vessels [36]. Also, different subsets of tumor-associated macrophages coexist in different tumor microenvironments [35]. For example, M1-like, or classically activated, macrophages secret cytokines such as interleukin 6 (IL-6), IL-12, and tumor necrosis factor α (TNF-α), etc. They produce reactive oxygen species (ROS). Thus, M1-like macrophages are generally pro-inflammatory, pro-immunity, and anti-tumor [34]. On the other hand, M2-like, or alternatively activated, macrophages secrete cytokines such as IL-10, IL-1β, transforming growth factor β (TGF-β), matrix metalloproteinase (MMPs), etc. Therefore, M2-like macrophages are predominately anti-inflammatory, immunosuppressive, pro-angiogenic, and

pro-tumor [34]. Neutrophils serve as a host's first defense against invading microorganisms through their attraction to the primary site and their contribution to tissue repair [37]. They can, however, infiltrate the tumor microenvironment to become tumor-associated immune-suppressive neutrophils and secret cytokines such as IL-1β, arginase-1, MMPs, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), etc [37, 38].

The immunomodulatory effects of berry compounds on cultured cells have been extensively summarized in other reviews [1, 39], and will not be repeated here. This review focuses on immunomodulation by phytochemicals found in berries, berry extracts, or whole berries as shown in tumor-bearing animal models (Table 1) and human studies (Table 2).

3. Studies of tumor-bearing animal models

3.1. Black raspberries

Black raspberries (BRBs) are rich in anthocyanins (AC), which gut bacteria metabolize into protocatechuic acid (PCA). Our group investigated the ability of BRBs, BRB components (such as AC), and BRB metabolites (such as PCA) to inhibit N-nitrosomethylbenzylamine (NMBA)-induced esophageal cancer and to modulate immune cell trafficking in rats [40]. All rats were injected with NMBA and then they were fed with either a control AIN-76A diet, or the control diet supplemented with either 6.1% of BRB powder (freeze-dried whole BRBs), an AC-rich fraction of BRBs (3.8 μmol/g), or PCA (500 ppm) for 35 weeks. In comparison with the control diet, all three study diets (BRB powder, AC, and PCA) suppressed tumor development in the esophagus of the NMBA-treated rats. In addition, all three study diets increased the levels of IL-10 and IL-12 in the plasma. More importantly, all three diets also decreased infiltration of both macrophages and neutrophils into the esophagus. These results suggest that increased IL-12 expression could activate cytolytic NK and $CD8^+$ T cells to kill tumor cells, while decreased infiltration of macrophages and neutrophils may reverse the immune-suppressing tumor microenvironment. However, the effect of higher IL-10 expression inside the tumor microenvironment warrants further investigation.

With regard to colon cancer, our group used $Apc^{Min/+}$ mice as an animal model of colon cancer [41] and fed them with either a control AIN-76A diet or the control diet supplemented with 5% BRBs for 8 weeks. We observed that 5% BRBs suppressed colon polyp development in $Apc^{Min/+}$ mice [41]. The percentage of GR-1^+ neutrophils and their cytokine secretion (IL-1β) were increased in colonic lamina propria, but decreased in colon polyps of $Apc^{Min/+}$ mice by BRBs, suggesting that BRBs may reverse the immune-suppressing tumor microenvironment in the $Apc^{Min/+}$ mice. In addition, the ability of BRBs to modulate NK cells was examined in two mouse models of colon cancer [42]. We gave the $Apc^{Min/+}$ mice and the azoxymethane (AOM)-injected mice 5% dextran sulfate sodium (DSS) in the drinking water overnight, in order to avoid the DSS-induced excessive inflammation that leads to colitis. This approach only slightly irritated the colon but still promoted colon carcinogenesis, with 100% incidence in both models. Four-week administration of 5% BRBs significantly suppressed colon cancer progression and increased the number of tissue-infiltrating NK cells, which might lead to their enhanced cytolytic killing of tumor cells.

3.2. Red raspberries

Polysaccharides are a class of natural macromolecules. One group investigated the anti-tumor effects of red raspberry pulp polysaccharides (RPP) in a mouse model of melanoma [43]. The yield of polysaccharide in the red raspberry pulp in Qinghai plateau is up to 12%, which provides a rich source for the extraction of polysaccharides. B16F10 mouse melanoma cells were implanted subcutaneously into C57BL/6 mice. These mice were given RPP orally at a dose of 100, 200, or 400 mg/kg of body weight for 2 weeks. RPP significantly

Table 1
Immunological properties of berries and their phytochemicals in tumor-bearing animals

Berries or berry components	Doses and Routes	Cancer types	Animal models	Immunological effects	Ref.
Black raspberries (BRBs)	6.1%, in the diet	Esophageal squamous cell carcinoma (ESCC)	N-nitrosomethylbenzylamine-induced ESCC in rats	IL-1β decreased	40
Anthocyanins (AC) from BRBs	3.8 mmol/g, in the diet			IL-10 and IL-12 increased	
Protocatechuic acid (PCA)	500 ppm, in the diet			Macrophages and neutrophils decreased	
BRBs	5%, in the diet	Colon cancer	ApcMin/+ mice	GR-1$^+$ neutrophils and IL-1β increased in colonic lamina propria GR-1+ neutrophils and IL-1β decreased in colon polyps	41
			ApcMin/+ mice treated overnight with DSS Azoxymethane (AOM)-treated mice treated overnight with DSS	Tissue-infiltrating NK cells increased	42
Red raspberry pulp polysaccharides	100, 200, 400 mg/kg daily × 2 weeks, oral	Melanoma	Mouse B16F10 melanoma cells in C57BL/6 mice	TNF-α, IFN-γ, IL-2 increased	43
Laricitrin	30 mg/kg daily × 3 days, i.p.	Lung cancer	Mouse Lewis lung carcinoma cells in C57BL/6 mice	IL-10 decreased in dendritic cells IL-12 increased in dendritic cells IFN-γ increased in CD4$^+$ T cells IL-4 and IL-5 decreased in CD4+ T cells	44

(Continued)

Table 1
(Continued)

Berries or berry components	Doses and Routes	Cancer types	Animal models	Immunological effects	Ref.
Grape seed proanthocyanidins	200 mg/kg daily × 10 days, oral	Sarcoma	Mouse sarcoma S180 cells in BALB/c mice	NK cell cytotoxicity increased IL-2 and INF-γ increased	47
Grape antioxidant dietary fiber from red grape pomace	1%, in the diet	Colon cancer	ApcMin/+ mice	Alteration of genes associated with the immune response	48
Resveratrol	10 mmol/mouse, topical	Skin cancer	Dimethylbenz(a)anthracene (DMBA)-induced skin cancer	IFN-γ and IL-12 levels increased	49
	12.5, 25, 50 mg/kg daily × 3 weeks, oral	Lymphocytic leukemia	Mouse L1210 lymphocytic leukemia in BALB/c mice	IL-6 decreased	50
	4 mg/kg × 1 dose, i.p.	Lymphoma	Mouse lymphoma EG7 cells in C57BL/6 mice	CD4/CD8 ratio increased $CD4^+$ $CD25^+$ T regulatory cells decreased TGF-β decreased IFN-γ increased in $CD8^+$ T cells	51
Polysaccharide-rich substance (noni-ppt) from noni	0.5 mg/mouse × 4-5 doses, i.p.	Sarcoma ascites tumor	Mouse sarcoma 180 cells in DBA/2, C57BL/6, and BALB/c mice	Requires functional macrophages, T cells and NK cells Needs IFN-γ	53
Fermented noni exudate	500 mL/mouse daily × 3 days. i.p.	Sarcoma	Mouse sarcoma 180 cells in C57BL/6, nude, and beige mice	Granulocytes and NK cells increased Requires functional NK cells and lymphocytes	54
M. alba L. fruit extract (white mulberry)	100, 300 mg/kg, in the diet	Colon cancer	Mouse CT26 cells in BALB/c mice	NK cell activity, cytotoxic T lymphocyte activity, and IFN-γ increased	55

Table 2
Immunological effects of berries in humans

Berry fruits	Intake amount	Human populations	Immunological effects	Ref.
Black raspberries	60 g freeze-dried berries daily for 1–9 weeks	Colon cancer patients	The number and cytotoxicity of NK cells increased	42
Various fruits such as grape, strawberry, raspberry, currant, blueberry, apple, and cherry	Servings in questionnaire	General population	No overall association with the risk of lymphoma	59
Fruit juice, including apple juice, orange juice, grape juice, prune juice, and other juice	Servings in questionnaire	Nurses Health professionals	Lower EDIP scores	60
Prudent dietary pattern	High intakes of vegetables, fruits, whole grain products; low intakes of refined grain products	Healthy individuals	A protective effect against cancer initiation or development	61
A tomato-based drink (Lyc-o-Mato)	Lyc-o-Mato drink daily containing 5.7 mg of lycopene, 3.7 mg of phytoene, 2.7 mg of phytofluene, 1 mg of beta-carotene, and 1.8 mg of alpha-tocopherol for 26 days	Healthy individuals	TNF-α decreased	62
Tomato oleoresin extract capsules	Tomato oleoresin extract capsules daily containing 14.64 mg lycopene, 1.44 mg phytoene, 1.32 mg phytofluene, and 3.543 mg alpha-tocopherol for 2 weeks	Healthy individuals	IL-4 decreased in smokers	63
Blueberry-apple juice	One liter drink daily providing 97 mg quercetin and 16 mg ascorbic acid for 4 weeks	Healthy individuals	Alteration in many pathways	64

inhibited the tumor growth with an inhibition ratio of 7.56%, 24.32% and 59.95%, respectively. In addition, RPP significantly increased the levels of TNF-α, IFN-γ, and IL-2 in the serum of the tumor-bearing mice in a dose-dependent manner. The increased secretion of TNF-α and IFN-γ might link to a stronger anti-tumor immune response by T cells and macrophages, which warrants further determination.

3.3. Grapes

Laricitrin, a flavonoid found in grapes, was evaluated for its ability to modulate the immune system in mice bearing lung tumors [44]. Lewis lung carcinoma (LLC) cells were implanted into male C57BL/6 mice via tail vein injection. The mice were intraperitoneally (i.p.) injected daily with either normal saline or laricitrin at a dose of 30 mg/kg of body weight, which was equivalent to the pharmacokinetics of grape phenols. Three doses of laricitrin decreased IL-10 levels and increased IL-12 levels in DCs from the tumors. IL-10 has shown to prevent the differentiation and maturation of DCs from monocytes, and impair the antigen-presenting function of DCs [45]. Thus, the increased ratio of IL-12/IL-10 in the tumor microenvironment by laricitrin might enhance the tumor-destructive Th1 response of T cells against LLC cells. In addition, laricitrin increased IFN-γ levels, and decreased IL-4 and IL-5 levels in $CD4^+$ T cells from the tumors, suggesting a switch from a Th2 response (IL-4 and IL-5 release) to a Th1 response (IFN-γ secretion) in $CD4^+$ T cells by laricitrin treatment. These results indicate that laricitrin may suppress LLC tumor growth through stimulating the anti-tumor Th1 response of T cells.

Grape seeds are a rich source of proanthocyanidins, the major polyphenols in red wine [46]. The abilities of grape seed proanthocyanidins to inhibit tumor growth and modulate immune system have been examined in mice bearing the mouse sarcoma 180 cells [47]. Female BALB/c mice were inoculated subcutaneously with the sarcoma 180 cells. The mice were orally given either normal saline or grape seed proanthocyanidins at a dose of 200 mg/kg of body weight daily for 10 days. This study found that grape seed proanthocyanidins significantly suppressed the tumor growth, increased the cytotoxicity of NK cells, and stimulate their secretion of IL-2 and IFN-γ in splenic lymphocytes. More importantly, when combining grape seed proanthocyanidins with doxorubicin, an anthracycline antibiotic in cancer therapies, grape seed proanthocyanidins strongly enhanced the anti-tumor effects of doxorubicin through reversing the immune-suppressive side effects that were caused by doxorubicin. These results suggest a potential combination of immune-therapeutic regimen of grape seed proanthocyanidins and doxorubicin.

Dietary fiber is another rich source of proanthocyanidins. One group treated $Apc^{Min/+}$ mice either a control diet (Teklad Global 18% Protein rodent diet), or the control diet supplemented with 1% grape antioxidant dietary fiber (GADF), a lyophilized red grape pomace containing proanthocyanidins-rich dietary fiber, for 6 weeks [48]. This study found that GADF suppressed the intestinal tumor development, and downregulated several genes associated with the immune response, such as CXCR4 signaling, which has been reported to be involved in the tumorigenesis and lymph node metastasis.

Many studies have demonstrated the anti-tumor effects of resveratrol (trans-3,5,4-trihydroxystilbene), a polyphenol found in red grapes and in several other plant sources. One group pre-treated the C3H/He mice with 10 mmol/mouse resveratrol by applying it topically to the skin 1 hr prior to the exposure to dimethylbenz(a)anthracene (DMBA), a skin carcinogen [49]. Resveratrol significantly suppressed DMBA-induced skin tumorigenesis and angiogenesis. In addition, resveratrol increased the IFN-γ and IL-12 levels in skin lysate, which may stimulate the generation of IFN-γ-producing Th1-cells. These effects caused by resveratrol treatment were dependent on the function of the toll-like receptor 4 (TLR4).

Another group investigated the anti-tumor effects of resveratrol against leukemia [50]. Mouse lymphocytic leukemia cells L1210 were i.p. injected to male BALB/c mice, and then the mice were orally given either water or resveratrol daily at doses of 12.5, 25, and 50 mg/kg of body weight for 3 weeks. Resveratrol significantly prolonged the survival of these L1210-bearing mice in a dose-dependent manner. Interestingly, resveratrol significantly decreased the levels of intracellular IL-6 in a dose-dependent manner. Whether the lower IL-6 expression

associated with a decreased macrophage population needs further investigation. In addition, resveratrol (25 and 50 mg/kg, but not 12.5 mg/kg) increased the CD4/CD8 ratios in the peripheral blood. However, the function of these T cells in the peripheral blood and whether they directly contributed to the tumor inhibition remain unclear.

In a mouse model of lymphoma, mouse lymphoma EG7 cells were inoculated subcutaneously (s.c.) into female C57BL/6 mice [51]. Then these mice were given a single i.p. injection of either control or resveratrol at a dose of 4 mg/kg of body weight. Resveratrol decreased the percentage of $CD4^+$ $CD25^+$ T regulatory cells and levels of TGF-β in splenocytes, suggesting resveratrol could reverse the tumor-suppressing immune microenvironment. In addition, upon the treatment of resveratrol *ex vivo* to the cells from lymph nodes of the tumor-bearing mice, IFN-γ expression in $CD8^+$ T cells was increased, suggesting an enhanced cytolytic function of $CD8^+$ T cells against lymphoma cells.

3.4. Noni

Morinda citrifolia L. (noni) has been one of the most important traditional medicinal plants in Polynesia for more than 2000 years [52]. A polysaccharide-rich substance from the juice of noni fruit (Noni-ppt at a dose of 0.5 mg/mouse, 4–5 doses) produced a significantly higher cure rate in mice bearing sarcoma 180 ascites tumors [53]. Importantly, the observed anti-tumor activities of Noni-ppt were dependent on the function of macrophages, T cells, and NK cells, since administration of a specific inhibitor of each immune cell type could completely abolish the beneficial effects. In addition, the anti-tumor effects of Noni-ppt were also dependent on Th1 cytokine (IFN-γ), but not Th2 cytokines (IL-4 or IL-10). These results suggest an overall cytotoxicity-dominant immune status (Th1) was required for the protective effects of Noni-ppt against tumors.

Another group investigated the anti-tumor effects of fermented noni exudate (fNE) against sarcoma 180 tumors [54]. Three-day treatment of 500 μl/mouse/day fNE significantly increased the percentage of granulocytes and NK cells in the peripheral blood, peritoneum, and spleen. Importantly, NK cells play the most important role in fNE-produced anti-tumor effects, while functional lymphocytes partially contribute to the beneficial effects.

3.5. Other berries

Morus alba L. (white mulberry), found in Asia, has been traditionally used in Korea [55]. When orally given to BALB/c mice bearing CT26 colon tumors for 3 weeks, *M. alba* L. fruit extract (MFE) at a dose of 300 mg/kg of body weight, which was equivalent to 24.3 mg/kg human body weight, has been shown to significantly enhance the anti-tumor activity when in combination with 5-fluorouracil, and strongly promote NK cell activity, cytotoxic T lymphocyte activity, and IFN-γ production in spleen [55].

In summary, studies have examined potential abilities of modulating immune system in various animal models of cancer by berries and their phytochemicals. They have shown to promote the cytotoxicity of NK and $CD8^+$ T cells, as well as to boost IFN-γ secretion. However, the effects of berries on other immune cells, such as macrophages and neutrophils, and their cytokine production, such as IL-10, have not been consistently reported across studies. This could be attributed to the dual functions of these immune cells. The context of tumor microenvironment could shape the outcome of these immune cells, which may lead to different effects upon berry treatment.

4. Human studies

NK cells are an essential component of innate immunity against cancer development [56]. Our group investigated the effects of BRBs on NK cells in a pre-surgical window of opportunity trial in colorectal cancer patients. Twenty colorectal cancer patients consumed 60 g/day freeze-dried BRB powder for 1–9 weeks. Then biopsies of colorectal adenocarcinomas were collected before and after BRB consumption [57]. Using

immunohistochemistry, we demonstrated a significantly increased number of tumor-infiltrating NK cells (CD56) and enhanced cytotoxicity of these NK cells (CD107a) after BRB intervention [42].

Since very few human studies have directly examined the effects of berries on cancer patients' immune response, we also include some epidemiologic studies regarding cancer patients, as well as some berry-feeding studies involving healthy volunteers. One study determined the relationship between personalized dietary intervention and clinical measurements such as immune cell-mediated cytotoxicity in cancer patients [58]. Cancer patients, including those with pancreatic cancer, bile duct cancer, lung cancer, breast cancer, colon cancer, hepatocellular carcinoma, glioblastoma, ovarian cancer, cecal cancer, and osteosarcoma, received either a treatment-support diet if they were undergoing chemotherapy ($n = 10$), or a remission-support diet if they were in remission ($n = 10$) for 21–61 days [58]. Both diets were low glycemic, low fat, and high in plant protein. The treatment-support diet contained an additional 0.5 servings of protein. This study found an increased tendency in immune cell-mediated cytotoxicity in the treatment-support group. However, since regular meals, which contain garlic, onion, tomato, shiitake, rice bran, kale, blueberry, pineapples, and/or turmeric powder, were provided to all subjects, the potential effects of berries cannot be concluded.

Another study investigated the effects of diet on lymphomas, a heterogeneous group of malignant diseases of immune system cells [59]. The European Prospective Investigation into Cancer and Nutrition (EPIC) trial identified 849 lymphoma cases among 411,097 participants during a median follow-up of 6.4 years. This trial estimated fruit consumption data from validated dietary questionnaires [59], which includes various fruits such as grape, strawberry, raspberry, currant, blueberry, apple, and cherry. However, no overall association between total fruit consumption and the risk of lymphoma was detected.

Dietary patterns might be linked to colorectal carcinogenesis. They could affect systemic and local intestinal inflammation, and chronic inflammation interferes with the adaptive immune response [60]. Food frequency questionnaire (FFQ) data were collected from the databases of 2 prospective cohort studies: the Nurses' Health Study (since 1976) and the Health Professionals Follow-Up Study (since 1986) [60]. An empirical dietary inflammatory pattern (EDIP) score calculated based on FFQ data was used to correlate dietary patterns with colorectal carcinoma subtype. In particular, fruit juice, including apple juice, orange juice, grape juice, prune juice, and other juice, contributed to low EDIP scores. A higher EDIP score represented a pro-inflammatory dietary pattern. During the follow-up of 124,433 participants, 1,311 cases of colon and rectal cancer with available tissue data were documented. The association between the EDIP score and colorectal cancer risk was significant ($p_{trend} = 0.02$. Interestingly, the association varied based on the degree of peritumoral lymphocytic reaction ($p_{heterogeneity} < 0.001$). A higher EDIP score associated with an increased risk of colorectal cancer when the peritumoral lymphocytic reaction was absent or at a low degree ($p_{trend} < 0.001$). However, when the peritumoral lymphocytic reaction was intermediate or high, there was no risk of tumors. These results suggest an important role of the host immune response.

One cross-section study examined the associations between dietary patterns and gene expression profiles of healthy men and women [61]. Of 254 participants recruited from the greater Quebec City metropolitan area, 210 completed the study protocol. Dietary patterns were derived from a FFQ. RNA was extracted from peripheral blood mononuclear cells (PBMCs) from 30 fasting participants. The results identified two dietary patterns. The Prudent dietary pattern was characterized by high intakes of vegetables, fruits, and whole grain products, and low intakes of refined grain products. The Western dietary pattern was defined by high intakes of refined grain products, desserts, sweets, and processed meats. Both the dietary patterns induce gene changes in related with cancer, immune, and inflammation. Interestingly, the Prudent dietary pattern seems to have a protective effect against cancer initiation or development, while the Western dietary pattern has an opposite effect.

Consumption of tomatoes and their products at a regular basis has been shown to associate with a lower risk of several types of cancer [62]. In a placebo-controlled, double-blind, crossover study, 26 healthy young volunteers (age < 30 years) drank either a tomato-based drink (Lyc-o-Mato) (containing 5.7 mg of lycopene, 3.7 mg of phytoene, 2.7 mg of phytofluene, 1 mg of beta-carotene, and 1.8 mg of alpha-tocopherol) or a placebo drink for 26 days [62]. Meanwhile during the study, they maintained their original habitual diet. TNF-α levels in

the whole blood were 34.4% lower in the subjects who drank Lyc-o-Mato. Another double-blinded, randomized, placebo-controlled study determined whether 2-week consumption of a tomato oleoresin extract affected immune functions of peripheral blood lymphocytes in healthy nonsmokers and smokers [63]. Fifteen nonsmokers and 12 smokers were given three capsules of tomato oleoresin extract daily, with each capsule containing 4.88 mg lycopene, 0.48 mg phytoene, 0.44 mg phytofluene, and 1.181 mg alpha-tocopherol. Tomato oleoresin extract significantly reduced IL-4 production in smokers, similar to the level found in nonsmokers. These studies suggest a potential anti-inflammatory effect of tomato.

The concept of the exposome in a human nutrigenomics study involving in-depth analyses of gene expression responses was applied in a dietary intervention trial with blueberry-apple juice [64]. For 4 weeks, 168 healthy volunteers consumed 1 liter of a custom-made blueberry-apple juice mixture every day, which provided 97 mg quercetin and 16 mg ascorbic acid. Blood collected before and after the intervention was used for plasma and lymphocyte analyses. The results showed that many pathways were altered in lymphocytes by the consumption of blueberry–apple juice. For example, in the NF-κB pathway, *RELB, IKK*β and *IKK*γ were upregulated, whereas *IKK*α was downregulated. In the JAK/STAT pathway, *JAK1* and *STAT3* were downregulated, whereas *JAK2, JAK3, STAT1,* and *STAT6* were upregulated. The juice also modulated genes involved in innate and adaptive immunity that are important for inducing anti-tumor immune responses.

In summary, berry intervention studies in humans involved fresh fruits and vegetables, juice, and freeze-dried powder. Encouragingly, our group demonstrated that BRBs increased the number and function of NK cells of colorectal cancer patients. However, very few studies directly examined the immune-modulating effects of berries on cancer patients. Although some large epidemiologic studies show that berry consumption might contribute to a lower risk of developing cancer, these studies usually categorize food items into several big groups, and berry is not an independent group. Therefore, the findings from epidemiologic studies have mixed results. Furthermore, berries could modulate genes involved in both innate and adaptive immunity in healthy individuals, which are important for inducing anti-tumor immune responses. We also need to consider that healthy volunteers can have very different immune system compared to that of cancer patients, so that any observations in healthy volunteers may not be able to translate into cancer patients. Therefore, much more clinical studies are needed to investigate the potential of berries and their components on the cancer immunity, as well as the mechanisms of their actions.

5. Conclusions

The literature shows some evidence that berries and their phytochemicals could modulate immunity to delay cancer development and progression. The wide range of phytochemicals in berries possibly explains the diversity of their effects on immune cells and cancer immunity. Recently, cancer immune-therapies, which depend on T cell recognition of tumor cells, have generated intense interest because of their success in treating some cancers [28]. The potential of berries and their phytochemicals to aid cancer immune-therapies—by regulating DCs, for example—warrants investigation beyond laboratory studies. In addition, more effort is needed to investigate the effects of berries and their phytochemicals on the entire spectrum of cancer immunity to provide a comprehensive picture of how they could be used to modulate immunity during cancer prevention and treatment.

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