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Citrus Flavonoids and Human Cancers

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Abstract Nowadays cancers pose a great threat to the health of human beings. Based on studies on the flavonoids and other bioactive compounds of *Citrus* fruits in current literature, it was widely suggested that the consumption of *Citrus* fruits is beneficial to the prevention and treatment of human chronic diseases including cancers. In the past decades, the study concerning *Citrus* flavonoids has covered various areas including the type, content and distribution of flavonoids in fruits; their variation between wild and cultivated genotypes; their antioxidant, anti-inflammation, anti-aging, antimicrobial and anticarcinogenic activities. To enlighten future *Citrus* germplasm study, this review introduces briefly the functions of main types of *Citrus* flavonoids, including antioxidant, anti-inflammation and anti-aging activities, and their relationships to human cancers. Most importantly, the mechanisms of action by which *Citrus* flavonoids play their roles in human cancer prevention and treatment were summarized.

Keywords: *Citrus fruits, antioxidant, anti-inflammation, anti-aging, human cancers, mechanism of action*

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1. Introduction

Cancers as the most common death-causing diseases have posed a great challenge to human health safety in current society. It is widely known that the consumption of fresh fruits including *Citrus* fruits is beneficial to the human health, especially to the prevention and treatment of chronic diseases [1,2]. *Citrus*, the genus *Citrus* L., Rutaceae family, is one of the most widely cultivated fruit crops in the world. *Citrus* fruits and their derived products have attracted much attention of consumers for their special flavor, color, nutritional and health-promotion values. They are gradually become an important part of our daily diet.

In the past decades, the health-promotion effects of *Citrus* fruits have been studied by researchers of multidisciplines, mainly due to their rich functional components such soluble fibers, essential vitamins, mineral elements and bioactive compounds including flavonoids [3,4].

The aims of this review are: (1) to introduce the main flavonoids in *Citrus* fruits and their distribution and content variation in different fruit tissues and genotypes or species; (2) to summarize the current information on the bioactivity of *Citrus* flavonoids, their relationships to human cancers, and the mechanism of action by which *Citrus* flavonoids play their roles in prevention and treatment of different cancers.

2. Type, Content and Distribution of Flavonoids in *Citrus* Fruits

Flavonoids are a type of polyphenolic compounds, which typically have a structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. In plants, flavonoids comprise the most abundant type of polyphenols with more than 6000 compounds have been identified [5]. In *Citrus*, more than 60 *Citrus* flavonoids have been identified, which consist of mainly flavanones, flavones, flavonols and anthocyanins [6].

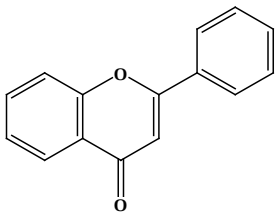

Figure 1. The general formula of flavonoids structure

2.1. Flavone

Flavones are a subgroup of flavonoids characterized by the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). In *Citrus* plants, 6, 8-di-C-β-glycosyldiosmin (LE-B) and 6-C-β- glycosyldiosmin (LE-C), luteolin, apigenin, chrysin, rhoifolin, quercitrin, luteolin, diosmetin, sinensetin, nobiletin and tangeretin were reported in existing literature.

The C-glucosyl flavones (LE-B and LE-C) were isolated and identified from the peels of 20 lemon fruits, and their contents reached 52.0 mg and 15.8 mg, respectively [7].

In recent study, it has been reported that seven flavones, i.e. rhoifolin, quercitrin, luteolin, diosmetin, sinensetin,

nobiletin and tangeretin were identified from the peels of 14 wild mandarins genotypes tested, among them nobiletin was found the most abundant one and the highest content was 6.83 mg/g dry weight (DW), followed by sinensetin and tangeretin, and the highest content was 4.71 mg/g DW and 2.92 mg/g DW, respectively [8].

Among the *Citrus* flavones, some of them were highly methoxylated, which were called polymethoxylated flavones (PMFs). The main PMFs found in *Citrus* are nobiletin, tangeretin and sinensetin.

Nobiletin, an important flavone component of *Citrus* fruits, was found mostly distributed in the flavedo and the highest concentration being found in Dancy tangerine, followed by that of Pankon, their concentration are 5.07 mg/g and 1.98 mg/g, respectively [9]. In the peels of mandarin fruits, the content of nobiletin ranged from 0.45 to 0.61 mg/g DW [10]. Recent, it was reported that nobiletin is the major PMFs in four grapefruit varieties compared with that of 28 pummelo, and the highest content is 43.71 mg/kg fruit weight (FW) [11]. Zhang et al. [8] found the content of nobiletin varied from 2.25 to 6.83 mg/g DW among the tested wild materials, and *Citrus reticulate* Blanco Wulongsuanju had the highest levels of it.

Tangeretin is other important PMF of *Citrus* origin. Bermejo et al. [10] reported that the tangeretin content in the peels of mandarin fruits ranged from 0.27 to 0.86 mg/g DW. Xi et al. [11] reported the tangeretin in the peels of *Citrus paradisi* cvs. Cocktail, Rio Red and Changshanhuyou are 30.21 mg/kg FW, 11.30 mg/kg FW and 34.75 mg/kg FW, respectively. While the highest content of tangeretin was found in the Changshanhuyou pulp and the content was 44.21 mg/kg FW. Moreover, Zhang et al. *[8]* reported that the content of tangeretin varied from 0.87 to 2.92 mg/g DW in the wild Chinese mandarins.

Sinensetin is a rare *Citrus* PMF found in certain types of *Citrus* fruits. It was reported that the sinensetin content in the peels of mandarin fruits ranged from 0.12 to 0.25 mg/g DW [10] Zhang et al. [8] reported that the content of sinensetin varied from 0.12 to 4.71 mg/g DW in the wild Chinese mandarins.

2.2. Flavonols

Flavonols are a type of flavonoids that have the 3-hydroxy flavone backbone (3- hydroxy-2-phenylchromen-4-one). In the current literatures, kaempferol, myricetin, quercetin and catechins were found the major *Citrus* flavonols.

Kaempferol is a flavonol found in many plants. Wang et al. [12] reported that kaempferol was the most abundant flavanol (0.009 ± 0.001–1.04 ± 0.007 mg/g edible fruits, DW) in fruits analyzed and the highest content was found in Murcott(C. reticulate×C. Sinensis) (1.035mg/g db). Zhang et al. [8] reported that kaempferol was found in four wild mandarin (*Citrus reticulata* Blanco) genotypes, namely, Daoxianyeju, Nieduyeju, Cupigoushigan and Jizigan. Their content were 0.08, 0.1, 0.08, 0.03mg/g DW, respectively. Myricetin is an another natural flavonol. Protti et al. [13] found that the total myricetin of Chinotto fruit (*Citrus* × *myrtifolia*) was higher than those of exocarp and endocarp, the value was about 3.84 ± 0.05 μg/g.

Quercetin is a flavonol widely found in many fruits. It was reported that among eight *Citrus* fruits studied by Wang et al. [7], lemon had the highest quercetin content (0.573 mg/g db).Wang et al. [9] reported that the contents of quercetin in the eight *Citrus* peels varied from 0.14 to 0.78 mg/g db. Zhang et al. [8] studied the quercetin content in six wild Chinese mandarins (Dakengyeju, Nieduyeju, banyeshengjuzi No.2, Tugan, Jinju, Xichuanzhoupigan), and found that their content varied from 0.07 to 0.86 mg/g DW. Protti et al. [13] found that the quercetin content in exocarp of Chinotto fruit (5.14 ± 0.04μg/g) was higher than those of whole fruit and endocarp [13].

In addition, Protti et al. [13] reported that the content of catechins in Chinotto whole fruit, exocarp and endocarp were 0.72±0.05, 0.39±0.03, 0.31±0.03μg/g, respectively.

2.3. Flavanone

Flavanones are the flavonoids characterized by glycosylated by a disaccharide at position seven to give flavanone glycosides. Flavanones occur almost exclusively in *Citrus* fruits, and *Citrus* flavanones are present in aglycone or the glycoside forms. Up till now, naringenin/naringin, narirutin, hesperetin/hesperidin, neohesperidoside, neohesperidin and neoeriocitrin are the major flavanones reported in *Citrus*.

Different *Citrus* flavanones are present in different species. Naringenin can be found as glycosides, such as naringin and narirutin, are especially abundant in *Citrus* fruits. For examples, naringenin is the most prevalent flavanone in grapefruits (*Citrus* paradisi) [9]. Kawaii et al. [14] reported that naringin (naringenin 7-O-neohesperidoside) is distinctly dominant in grapefruit, accompanied by narirutin (naringenin 7-O-rutinoside). Nogata et al. [9] reported that the highest content of naringin was found in albedo tissue of grapefruit, and the value was 27mg/g FW. While the lowest naringin concentration was found in flavedo tissue of grapefruit and the value was 9.94mg/g. In the wild mandarins, the highest content of naringenin, naringin and narirutin were found in Cupigoushigan, Wangcangzhoupiga and Cupigoushigan. The values were varied 0.49, 4.28, 6.89 mg/g DW, respectively [8]. Hesperetin is another flavanone mainly exist in *Citrus* fruits. Hesperetin is the most prevalent flavanone from oranges. it was found in the fruit tissue and peel largely as its glycosides, hesperidin and neohesperidin, which are conjugates with rutinose and neohesperidose, respectively [9]**.** Hesperidin, the 7-rutinosides of hesperetin, found in lemon peel and the concentration was 1.73mg/ml [15]. Significant amounts of hesperidin also occur in grapefruits, in which the highest content of hesperidin in grapefruit was 3.95mg/100g, while tangelo and sour orange are especially rich in neohesperidin [16,17]**.** Besides**,** Wang et al. [3] reported that the high content of hesperidin was found in the peel of Ponkan and the value was 29.5 mg/g db, while the neohesperidin was found in peel of Wendun(Citrus. Grandis Osbeck), and the content was 0.34 mg/g, db. In the wild Chinese mandarins, hesperidin was the major flavanone, and the highest content 55.98 mg/g DW was found in Guangxihongpisuanju (*Citrus reticulata* Blanco) [8].

Eriocitrin, the 7-rutinosides of eriodictyol, is particularly abundant in lemon, while they are almost

absent in some other *Citrus* fruits such as kabosu (*Citrus. sphaerocarpa*) and pink grapefruit (*Citru. paradisi*)**.** The highest of concentration of eriocitrin is 285 mg/ 100 ml from pulp vesicles of lemon fruits [15]. Eriocitrin was also found in the wild mandarins, the contents of it varied from 0.79–8.51 mg/g DW [8].

In addition, the flavanones varied in different organs/tissues of *Citrus* species. Bocco et al. [18] reported that the lemon seed mainly contains eriocitrin and hesperidin, while the peel is rich in neoeriocitrin, naringin and neohesperidin. The glycosylated flavanone, neoeriocitrin and naringin have similar concentrations in peel while, in seed, eriocitrin is 40 times more abundant than is naringin**.**

2.4. Anthocyanins

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. They belong to a parent class of molecules called flavonoids synthesized via the phenylpropanoid pathway [19]. The basic chemical structure of anthocyanin is shown in Figure 2-D. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. In *Citrus*, anthocyanins were studied in blood oranges [20]. They were found in the epicarp, but they also colour the mesocarp of oranges. The major anthocyanins of the juice of pigmented oranges were characterized as cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside) [21,22]. Kelebek et al. [23] found that cyanidin 3-(6″-malonyl glucoside) and cyanidin 3-glucoside were the most dominant anthocyanins in two blood orange varieties [Moro and Sanguinello (*Citrus sinensis* (L.) Osbeck)]. Further, Moro and Sanguinello juice had 291.3 and 43.07 mg/l of anthocyanins, respectively. In another study, six minor anthocyanins were reported in blood orange juices by Hillebrand et al. [21], i.e., cyanidin 3,5-diglucoside, delphinidin 3-glucoside, cyanidin 3-sophoroside, delphinidin 3-(6″-malonylglucoside), peonidin 3-(6″-malonylglucoside), and cyanidin 3-(6″-dioxalylglucoside). The anthocyanin content is strongly dependent on the level of maturation of fruits [24].

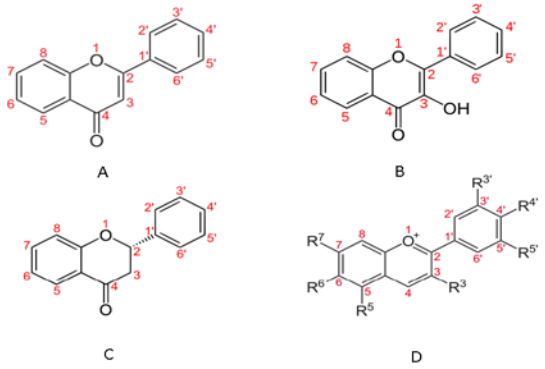

Figure 2. The basic structure of four *Citrus* flavonoids. (A) Molecular structure of the flavone backbone with numbers. (B) Backbone of a flavonol, substituent numbers are indicated (C) Flavanone skeleton with locants. (D) The basic chemical structure of anthocyanins

3. Bioactivity of *Citrus* Flavonoids and Their Relationship to Human Cancers

In current literature, *Citrus* flavonoids have been reported to have a wide range of biological functions from antioxidant, anti-inflammatory, anti-aging, antifungal to antimutagenic activities [25,26]. Among these, the antioxidant, anti-inflammatory and anti-aging activities have been the most widely studied and suggested to be closely associated with cancers of human beings [27,28].

3.1. Antioxidant Activity and Cancer

3.1.1. Antioxidant Activity *Citrus* Flavonoids

An antioxidant can be defined as a compound that inhibits or prevents oxidation of a substrate [29]. Antioxidant activity denotes the ability of a bioactive compound to maintain cell structure and function by effectively clearing free radical, inhibiting lipid peroxidation reaction, and preventing other oxidative damages [30]. *Citrus* flavonoids mainly function as reducing agents and metal chelators, reactive oxygen species (ROS) scavengers, chain-breaking antioxidants, quenchers of singlet oxygen formation, and protectors of ascorbic acid [31]. These characteristics contribute to prevent the propagating chain reactions of these oxygen free radicals [25]. A wide range of studies had demonstrated the antioxidant activity of *Citrus* [31,32]. Xi et al. [11] and zhang et al.[8] reported that *Citrus* flavonoids can scavenge free radicals and the superoxide anion, reduce the ferric complex to the ferrous form. *Citrus* flavonols, such as quercetin and kaempferol, were reported to possess significant antioxidant activities [25].

Citrus flavanones including eriocitrin, neoeriocitrin, hesperidin, hesperetin, neohesperidin, naringenin, and naringin, have been reported to have moderate antioxidant activities. Eriodictyol isolated from lemon (*Citrus limon*) juice exhibited a potent radical scavenging activity and superoxide [33]. Usually, the antioxidant activities of *Citrus* glycosides are weaker than those of the aglycons; because glycosides must be hydrolyzed to their corresponding aglycones before absorption [30]. However, the inhibition of hesperidin on the formation of R• and polyunsaturated fatty acid-derived free radicals was better than that of hesperetin [34,35]. Wilmsen et al. [36] reported that *Citrus* flavonoid hesperidin have the ability of scavenging radicals, and it can dose-dependently inhibit the Cu^{2+}-induced oxidation of oxidation of low density lipoprotein (LDL) *in vitro*, promote pancreatic B cells regeneration, and prevent and treat the oxidative stress on the embryos of diabetic pregnant rats [37].

Naringin can significantly enhance the immune system's effectiveness to avoid internal organs and tissue injury or disease caused by oxidation reaction through increasing the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), paraoxonase (PON) and other antioxidant enzymes [38]. Naringenin can inhibit β oxidation of fatty acids in the liver by regulating key enzymes in fatty acid oxidation process activity, including glucose-6-phosphate dehydrogenase (G6PD), phosphatidic acid phosphate hydrolase (PAP, triglyceride synthesis rate-limiting enzyme), fatty acid synthase (FAS), carnitinepalmitoyl transferase(CPT, the rate-limiting enzyme of fatty acid oxidation),3-hydroxy-3-methyl-glutaryll-CoA reductase, acyl-coenzyme A-cholesterol acyltransferase (ACAT), paraoxonase (PON) and plasma antioxidant enzymes, etc [39].

3.1.2. Antioxidant Activity and Cancer

Oxidative stress is a disturbance in the balance between the production of ROS and antioxidant defenses. Oxidative stress is known to cause DNA damage and mutations of tumor suppressor genes that are critical initial events in carcinogenesis [40]. Commonly, sources of internal oxidative stress include peroxisomes and enzymes, particularly the detoxifying enzymes from the P450 complex, xanthine oxidase, and the nicotinamide adenine dinucleotide (NADPH) oxidase complexes, which include the NADPH oxidase (Nox) family. Most of these enzymes act in the mitochondria, which is the main source of oxidative stress. External sources of oxidative stress include UV radiation, chemical compounds (e.g., environmental pollutants, smoking), and exercise.

Cancer is a multistage process defined by at least three stages: initiation, promotion, and progression [41,42]. Oxidative stress interacts with three stages of this process. During the initiation stage, ROS may produce DNA damage by introducing gene mutations and structural alterations into the DNA. In the promotion stage, ROS can contribute to abnormal gene expression, blockage of cell-to-cell communication, and modification of second-messenger systems, thus resulting in an increase in cell proliferation or a decrease in apoptosis of the initiated cell population. ROS have been shown to modulate cell cycle regulation through modulation of various cell cycle proteins such as p53 [43]. Finally, oxidative stress may also participate in the progression stage of the cancer process by adding further DNA alterations to the initiated cell population [44].

3.2. Anti-inflammation Activity and Cancers

3.2.1. Anti-inflammation Activity of *Citrus* Flavonoids

Inflammation is a part of the non-specific immune response that occurs in reaction to any type of bodily injury [45]. Inflammation is produced by immune cells within the tissue, which release specific mediators that control local circulation and cell activities. The internal and external stimuli that cause anti-inflammation responses are widespread, including microbial and viral infections; exposure to allergens, radiation, and toxic chemicals; autoimmune and chronic diseases; obesity; consumption of alcohol; tobacco use; and a high-calorie diet [46,47].

Different inflammation mediators have different influences on the inflammation cascade. Typically, an anti-inflammatory agent exhibits therapeutic property by suppressing the action or synthesis of the inflammation mediators [48]. Flavonoids have been suggested to inhibit the key reactions catalyzed by phospholipase A2, cyclooxygenase (COX), and lipoxygenase in inflammatory responses [49,50]. These enzymes are involved in the syntheses of proinflammatory arachidonic acid derivates (AADs), such as prostaglandins E2, F2 (PGE2, PGF2) and thromboxane A2. These AADs are essential for activating neutrophils and thus stimulate the formation of ROS in inflammatory tissues [51,52]. *Citrus* flavonoids were found to affect the activation of a number of cells involved in the immune response, including T and B lymphocytes [58]. It was reported that oral administration of *Citrus* flavonoids can alleviate alcoholic liver disease (ALD) through preventing excessive lipid formation, suppressing induction of inflammation in hepatocytes [53]. Several clinical trials have also demonstrated the positive effect of *Citrus* flavonoids in the reduction of proinflammatory cytokines in humans [54,55].

The anti-inflammation activity of different *Citrus* flavonoids have also been investigated in the existing studies, such as hesperidin, diosmin, tangeretin, auraptene, naringenin, and quercetin. Quercetin has demonstrated significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation. For example, it inhibits both the production of histamine and other allergic /inflammatory mediators [15]. Hesperidin and diosmin were reported to have the anti-inflammatory property through inhibition of the synthesis and biological activities of different proinflammatory mediators, mainly the AADS, PGE2 and PGF2 and thromboxan A2 [48]. In addition, hesperidin and tangeretin extracted from tangerine peel (*Citrus reticulatae* pericarpium) were reported to have potent anti-neuroinflammatory capacity by suppressing LPS-induced proinflammatory NO, TNF-α, IL-1β and IL-6 secretion [56]. Moreover, tangeretin was reported to have markedly reduced LPS-stimulated phosphorylation of IκB-α and IKK-β, as well as the nuclear translocation of the p65 subunit of pro-inflammatory transcription factor NF-κβ [57]. Furthermore, auraptene (AUR) was reported to suppress the LPS-induced inflammation in the brain of model mice possibly through inhibiting the expression of (COX)-2 [58]. Naringenin was reported to have ameliorative effects on hyperglycemia-mediated inflammation in streptozocin (STZ) – nicotinamide induced DM in Wistar rats by decreasing the values of hematological, mRNA transcript and protein indices of inflammation [59]. Besides, Yoshida et al. [60]. found that in 3T3-L1 adipocytes cell culture, naringenin showed anti-inflammatory effect by inhibiting the activation of NF-κβ through TNF-a, with a consequent reduction in the secretion of interleukin-6 (IL-6); and antilipolytic effect by inhibit extracellular signal regulated kinase (ERK) pathway causing a decreased activation of hormone sensitive lipase (HSL); contributing to reduce the insulin resistance

3.2.2. Anti-inflammation Activity and Cancers

The link between inflammation and cancers is an old concern. As early as 1863, Virchow first noted that inflammatory cells are present within tumors and that tumors arise at sites of chronic inflammation [61].

In the past decades, although it was reported that acute inflammation contributed to the regression of cancer [62], an increasing number of studies support that chronic inflammation induced by biological, chemical, and physical factors is associated with an increased risk of several human cancers [63].

Chronic inflammation was reported to be linked to various steps of carcinogenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [64,65]. Chronic inflammation may contribute to cellular transformation through mutations, genomic instability, and epigenetic modifications. Also it activates tissue repair responses, induces proliferation of premalignant cells, and enhances their survival. Chronic inflammation stimulates angiogenesis, causes localized immunosuppression, and promotes the formation of a hospitable microenvironment in which premalignant cells

can survive, expand, and accumulate additional mutations and epigenetic changes. Inflammation also promotes metastasis [66]. For examples, inflammatory bowel diseases such as Crohn disease and ulcerative colitis are associated with increased risk of colon adenocarcinoma [67,68]. Up till now, different types of molecular players such as cytokines and chemokines were identified during the development from inflammation to carcinogenesis. These include the cytokines IL-6 and TNF-α, and the chemokines NF-κB, iNOS, COX-2, HIF-1a, STAT3, Nrf2 and NFAT. In addition, some proteins with extensive roles in inflammation and cancer, such as signal transducers and activators of transcription, nuclear factor of activated T cells, were proposed to be the promising targets for future chronic inflammation and carcinogenesis study [69].

3.3. Anti-aging Activity and Human Cancers

3.3.1. Anti-aging Activity of *Citrus* Flavonoids

Aging is a process of diverse detrimental changes in living organism with advancing age, resulting in an increase in the risks of disease and death, steady decline in many cognitive processes [70], particularly some types of memory [71]. The aging may be caused by internal genetic factor and many external factor, such as radiation, nutrition, smoking, alcohol, and environmental conditions [72,73].In addition, the ROS [72].

In the existing studies, many plant flavonoids have been reported to possess anti-aging activity. For example, Shakibaei et al. [74] summarized the anti-aging function of resveratrol, a most studied plant flavonoids rich in grape fruit, in yeast (*Saccharomyces cerevisiae)*, the fruit fly (*Drosophila melanogaster)*, the nematode worm (*Caenorhabditis elegans)*, and seasonalfish *(Nothobranchius furzer)*.In addition, quercetin has been reported to have preventive effect against aging through decreasing oxidative stress markers, namely, levels of ROS, glutathione oxidation, protein carbonylation, and lipid peroxidation [75].In respect to the *Citrus* flavonoids, hesperidin was reported to have anti-aging activity in current literature. *In vivo* study by Sun et al. [76]reveled that hesperidin can extend the lifespan of yeast. They found that hesperidin can significantly inhibit the ROS, the SIRT1 activity, *UTH1* and SOD gene expression of yeast, and that SKN7 gene is involved in hesperidin-mediated lifespan extension.

3.3.2 . Anti-aging Activity and Cancers

For most species, aging promotes a host of degenerative processes that are characterized by debilitating losses of tissue or cellular function, especially among vertebrates, aging also promotes hyperplastic pathologies, the most deadly of which is cancer [77]. It was reported that cancer incidence rises exponentially with age in humans and most other mammals [78]. Unfortunately, aging and cancer research remain largely separate endeavors in the past decades. However, it is gradually accepted that age is the largest single risk factor for developing cancer, and that aging and cancer are linked by intriguing and complex biology, certainly more than the simple passage of time to which the age-dependence of cancer has traditionally been ascribed. For example, there are a number of research findings and publications that reinforce both the strength and complexities of a biological relationship between cancer and aging [79].

4. *Citrus* Flavonoids and Human Cancer Prevention and Treatment

In current society, cancers have already become a serious social issues. There are over 100 cancers that seriously affect humans beings, among them, the most common ones are lung cancer, prostate cancer, colorectal cancer, and stomach cancer of male people, and the breast cancer, colorectal cancer, lung cancer, and cervical cancer of female people [80]. A wide array of both internal factors, such as gene mutations, changes in hormonal and immune systems, and metabolic abnormalities, and external factors such as lifestyle choices like excessive alcohol consumption, unhealthy diet, exposure to excessive sunlight and carcinogenic chemicals, lack of exercise, and cigarette smoking were suggested to be associated with carcinogenesis [81]. Unfortunately, the precise cause of cancer is still largely unknown.

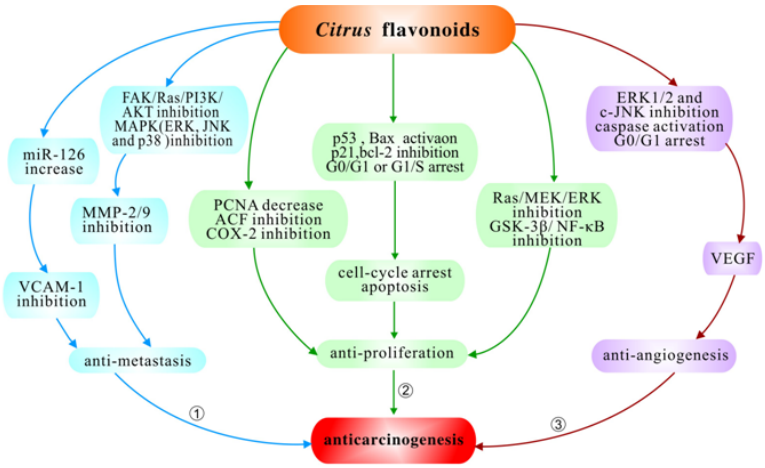

Figure 3. A schematic representation of the anticarcinogenic pathways of *Citrus* flavonoids. (1) The anti-metastasis activity via inhibition of VCAM-1 and MMP-2/9 by increasing miR-126, and attenuating the FAK/Ras/PI3K/AKT and MAPK signaling pathways. (**2**) The anti-proliferation activity via decreasing PCNA or inhibiting the formation of ACF or inhibiting the expression of COX-2; via cell-cycle arrest and apoptosis by inducing cell-cycle arrest at the G0/G1 or G1/S phase , activing p53 and Bax and inhibiting p21 and bcl-2; via inhibition of Ras/MEK/ERK and GSK-3β/ NF-κB signaling pathway. (**3**) The anti-angiogenesis activity via VEGF by inhibiting ERK1/2 and c-JNK, activating the caspase pathway or arresting cell-cycle at G0/G1

In the past decades, an increasing evidences have demonstrated that diet rich in vegetables and fruit is associated with a decrease in the incidence of various human chronic diseases such as cancers, obesity and diabetes, cardiovascular diseases, dementia and neurodegenerative disorders [2,82], and the studies concerning *Citrus* flavonoids and their roles in the prevention and treatment of human cancers have attracted more and more attentions in scientific world [83,84]. A summary of the possible mechanism of action by which *Citrus* flavonoids play their roles is given in Figure. 3. In the following, we focus on the anti-proliferation, anti-metastasis, and anti-angiogenesis activity of *Citrus* flavonoids.

4.1. Anti-proliferation

Proliferation refers to the cells which grow and increase in number rapidly in normal condition, but cancer cells proliferation are difficult to be controlled by body. Dysregulated proliferation appears to be a hallmark of susceptibility to neoplasia.

It has been suggested that flavonoids-rich diet has an adverse effect on the proliferation of cancer cells [94,95].Various mechanisms have been proposed to explain the anti-proliferative activity of flavonoids, for example, it has been proposed that the anti-proliferative effects of flavonoids are mainly mediated by the inhibition on several kinases and kinase inhibitors involved in cell-cycle arrest and apoptosis [85]. Cyclin-dependent kinases (CDKs) have been recognized as key regulators of cell cycle progression. Alteration and deregulation of CDK activity are pathogenic hallmarks of neoplasia [86]. Besides, the dysregulation of the checkpoints at both G1/S and G2/M of the cell cycle also play an important role in the development of malignant neoplasm [87]. *Citrus* flavonoids including apigenin, diosmin, rutin, tangeretin have been demonstrated to inhibit proliferation of different kinds of cultured human cancer cell lines including human squamous cell, carcinoma cell line (HTB43), MDA-MB-435 ER- human breast cancer cells, MCF-7 ER+ human breast cancer cells, DU-145 androgen receptor-negative human prostate cancer cells, HT-29 human colon cancer cells, DMS-114 human lung cancer cells, and SK-MEL5 human melanoma cells [83,84]. In the study of Kawaii et al.[88], twenty-seven *Citrus* flavonoids were examined for their anti-proliferative activities against tumor including lung carcinoma A549 and gastric TGBC11TKB cancer cells and normal human cell lines. The result showed that luteolin, natsudaidain, quercetin, tangeretin, eriodictyol, nobiletin, and 3,3′,4′,5,6,7,8-heptamethoxyflavone were suggested to be potential anti-cancer agents. In addition, Angst et al. [89] reported that quercetin caused significant apoptosis and reduced tumor cell proliferation in a nude mouse model. It was reported that the antiproliferative characteristics of hesperidin was inducing the expression and transcriptional activity of PPARγ and promoted p53 accumulation and downregulated constitutive NF-κB activity in a PPARγ-dependent and PPARγ-independent manner in NALM-6 cells [90]. Aranganathan et al. [91] also reported the antiproliferative effects of hesperetin. They found that supplementation with hesperetin (20mg/kg body weight) lowered the proliferating cell nuclear antigen (PCNA) labeling index and suppressed the formation of aberrant crypt foci (ACF) in the rats with colon cancer.

Nobiletin, tangeretin and sinensetin are the main PMFs found in *Citrus*. Lee et al. [92] revealed that PMF-mediated induction of *GADD45α* partially underlies the anti-proliferative effect of PMF on colorectal cancer cells. Nobiletin was found to act as an anti-carcinogenic compound through anti-proliferative activity, induction of apoptosis and cell cycle deregulation [93]. It was reported that tangeretin suppressed breast cancer proliferation by up-regulating p53/p21 proteins and inducing G1/S phase cell cycle arrest [94].

Glycogen synthase kinase-3β(GSK-3β) is phosphorylated by Akt, and GSK-3 itself is involved in the regulation of cell proliferation, anti-apoptotic pathways, and cell cycle progression [95]. Nuclear factor kappa B (NF-κB) transcription factors regulate several important physiologic processes of cell, e.g., cell growth, and apoptosis. Thus, inhibition of NF-κB activation offers a potential strategy for treatment of different malignancies [96]. Apigenin was found to induce pancreatic cell death through inhibition of GSK-3β/ NF-κB signaling pathway [97].

COX, an inflammatory enzyme induced by cytokines, catalyzes the conversion of AAD to PGEs. COX-2 expression in cancers is associated with tumor growth. Murakami et al. [98] reported that nobiletin showed the greatest anti-proliferative activity by suppressing the expression of COX-2 *in vitro*, and inhibiting dimethylbenz [1] anthracene (0.19 mmol)/TPA (1.6 nmol)-induced skin tumor formation. Ras, a small G-protein, physiologically directs cell proliferation and cell cycle via regulation of mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling cascade. Dysregulation of Ras/MEK/ERK signaling has been reported to cause tumorigenesis and gliomas. Aoki et al. [99] found that nobiletin suppresses the cell proliferation by inhibiting Ras activity and MEK/ERK signaling cascade probably *via* a Ca^{2+}-sensitive PKC-dependent mechanism. Hsiao et al. [100] also reported that nobiletin suppressed cell proliferation in HL-60 AML cells via inducing cell-cycle arrest at the G0/G1 phase by suppressing extracellular signal-regulated kinase (ERK) activity. In another study, the nobiletin was shown to inhibit the proliferation of human pancreatic cancer cells (PANC-1) by inducing apoptosis via up-regulation of the proapoptotic protein bax and down-regulation of the anti-apoptotic proteins bcl-2 and p53 [101].

17-β-estradiol (E2) is involved in the pathogenesis of several types of cancers. Bulzomi et al. [102] reported that naringenin can bind to ERα (estrogen receptor α) as an antagonist, thereby limiting the effect of E2 in promoting cellular proliferation.

4.2. Anti- metastasis

Metastasis is the spread of a cancer from its original site to new locations in the body. Among these the new tumors are called metastatic tumors, while the original is called the primary tumor. Most cancer deaths are due to the metastasis of the primary tumor [103].

In order to metastasize, tumor cells need to migrate and invade into the surrounding tissue, intravasation into the blood or lymph, circulation through the body,

extravasation into the new tissue, proliferation, and angiogenesis at a distant site [104]. The invasion of surrounding tissues by cancer cells involves several steps/stages, including matrix metalloproteinase (MMP) secretion, migration, invasion, and adhesion. MMPs are zinc and calcium-dependent proteases that digest most of the extracellular matrix components and are produced by cancer cells during invasion. They are secreted as latent proenzymes and are converted to the active form by proteolytic cleavage of an amino terminal domain [105].

In existing literature, *Citrus* flavonoids that have been reported to have anti-metastasis effects on tumors mainly through MMPs include the total flavonoids, naringin, hesperidin, kaempferol, tangeretin, naringin, naringenin, and nobiletin. Ishiwa et al. [106] reported that the flavonoids isolated from *Citrus depressa* Hayata including tangeretin, 6-demethoxytangeretin, nobiletin, 5-demethylnobiletin, 6-demethoxynobiletin, and sinensetin suppressed the interleukin 1 (IL-1) induced production of proMMP-9/progelatinase B in rabbit synovial cells in a dose dependent manner, among these flavonoids, nobiletin is most effectively in suppressing proMMP-9 production along with the decrease in its mRNA. Arivazhagan L et al. [94] reported that tangeretin treatment significantly suppressed matrix metalloproteinase (MMP)-2, MMP-9 in a DMBA-induced animal models. Lee et al. [107] found that naringin extracted from *Citrus* fruits including grapefruit suppressed the upregulation of MMP-9 and repressed the PI3K/AKT/mTOR signaling pathway. Naringenin were evaluated for its anti-metastasis effects by Lentini et al. [108]. They found that oral administration of naringenin to C57BL6/N mice inoculated with B16-F10 cells reduces the number of lung metastases. Park et al. [109] reported that *Citrus* flavonoids isolated from Korean *Citrus* aurantium L have anti-invasive effect through the inhibition of MMP-2 expression in A549 cells in a dose-dependent manner. Chen et al. [110] investigated the anti-metastatic activity of kaempferol and its molecular mechanism of action in human U-2 osteosarcoma (U-2 OS) cells. They found that kaempferol influenced the expression and enzymatic activities of MMP-2, MMP-9 and urokinase plasminogen activator (uPA) through attenuating the MAPK signaling pathways including ERK, JNK and p38 by decreasing DNA binding ability of AP-1.

Among the PMFs, tangeretin has been reported as a potential anti-metastasic agent. In human MCF-7/6 breast carcinoma cells, tangeretin treatment effectively inhibited the metastasis by down-regulating MMP-2 and MMP-9 in breast cancer [94]. In melanoma, the invasion of B16F10 cells was inhibited by the treatment of tangeretin [111]. Moreover, wound healing assay showed that tangeretin exerted inhibitory effects on SKOV3 cell migration. In addition, Lai et al. [112] that oral administration of Gold Lotion (GL), an extract of multiple varieties of *Citrus* peels containing abundant flavonoids, including a large percentage of PMFs, effectively suppressed the prostate cancer of human by mechanistic down-regulation of the protein levels of MMP-2 and MMP-9.

AKT, a serine/threonine protein kinase, is a downstream target of PI3K, and it plays a pivotal role in cell migration, growth, and anti-apoptotic events in various types of cells [113]. Seo et al. [114] reported that tangeretin inhibited platelet-derived growth factor-(PGDF-) BB-induced proliferation and migration of aortic smooth muscle cells by blocking AKT activation in a dose-dependent manner. The existing study indicated that the FAK/PI3K/Akt is involved in the regulation of MMP-2 and MMP-9 activities on different cell types [115]. In addition, NF-κB has been known to translocate to the nucleus and regulate the expressions of multiple genes involved in MMP-2/MMP-9 secretions. In AGS cells, nobiletin showed the inhibitory effect on the invasion and migration. The FAK/Ras/PI3K/AKT signaling pathway is a possible mechanism of the inhibitory effects of nobiletin on AGS cells, including the increased protein level of cytoplasmic IkB which exerts inhibitory effects on the transcriptional factor NF-κB, subsequently decresing MMP-2 and MMP-9 activities [116].

Hepatocyte growth factor (HGF), and its receptor, c-Met activation has recently been shown to play important roles in cancer invasion and metastasis in a wide variety of tumor cells. Besides, the activation of mitogen-activated protein/extracellular signal-regulated kinase (MEK/ERK) is well known to be associated with tumor invasion and metastasis. Shi et al. [117] reported that nobiletin attenuates HGF-induced HepG2 cells metastasis involving both ERK and PI3K/Akt pathways and are potentially useful as anti-metastatic agents for the treatment of hepatoma. In HT-1080 cells, nobiletin was also evidenced directly inhibited MEK activity and decreases the sequential phosphorylation of ERK, exhibiting the antitumor metastatic activity by suppressing MMP expression[118]. In respect to bone cancers, Tan et al. [119] reported naringin inhibits migration and invasion of human chondrosarcoma via down-regulation of vascular cell adhesion molecule-1(VCAM-1) by increasing miR-126.

4.3. Anti-angiogenesis

Angiogenesis is the establishment of the mature blood vessel network through expansion and remodeling of the preexisting vascular primordium, which is the base for solid tumour growth and dissemination, without a supply of new blood vessels, a tumor can only reach a small volume [120]. The tumor-associated neovasculature delivers nutrients and oxygen to tumours and removes their metabolic waste. In fact, angiogenesis is almost required at every step of tumor progression and metastasis [121].

The critical role of tumor angiogenesis in cancer progression was postulated in the 1970s [122]. Since then, there is growing evidence that angiogenesis plays an important role in the development of cancers [123]. Besides, MMPs have been shown to be tightly related to angiogenesis [124].

The process of angiogenesis is initiated by the dissolution of the endothelial basement membrane by proteinases which are responsible for weakening the tight contact of endothelial cells with the basement membrane and underlying mural cells, thus changing the phenotype of the endothelial cells, which become permissive to the activity of growth factors [125].Vascular endothelial growth factor-A (VEGF-A) is regarded as the major angiogenic factor during epithelial carcinogenesis in many malignant human cancers and in tumor metastases. *VEGF-A* gene encodes the ligand involved in neovascularization during the embryonic and neonatal development,

homeostasis, and survival of endothelial cells, as well as physiological and pathological state of adult [126].

Since angiogenesis is a pre-requisite for the growth of solid tumors, vascular targeting has been explored as a potential strategy to suppress tumor growth and metastasis. In this regard, *Citrus* flavonoids have been shown to target tumor angiogenesis. Lam et al. [127] reported that hydroxylated PMFs suppressed the expression of VEGF and MMP-9 in colonic tumors. Sinensetin, a common PMFs found in *Citrus* fruits, which showed the most potent anti-angiogenesis activity and the lowest toxicity, inhibited angiogenesis by inducing cell cycle arrest in the G0/G1 phase in HUVEC culture and downregulating the mRNA expressions of angiogenesis genes *fl1*, *kdrl*, and *hras* in zebrafish. Lam et al. [128] reported nobiletin inhibits angiogenesis by regulating cell cycle progression through G0/G1 arrest in vivo. Nobiletin also showed an antiangiogenic activity with the ID50 value being 10 lg (24.9 nmol) per egg in a chick embryo chorioallantoic membrane assay and inhibited angiogenic differentiation induced by VEGF and FGF by downregulation of ERK1/2 and c-JNK and activation of the caspase pathway. In addition, Arivazhagan et al. [94] reported that tangeretin treatment significantly altered expression of VEGF in DMB–induced animals, which confirms the anti-angiogenic potential of tangeretin.

5. Conclusion

It is well-accepted that *Citrus* phenolic compounds, especially flavonoids and PMFs have their roles in prevention and treatment of some human cancers. In current literature, the antioxidative and anti-inflammatory activities were suggested to be the main contributor to the functions of *Citrus* flavonoids. The exact cell and molecular mechanisms by which *Citrus* flavonoids play their roles in human cancer prevention and treatment still need further clarification. Future researches on *Citrus* flavonoids should focus on the bioaccessibility and bioavailability in vivo, such as developing a bio-system or method for precisely assessing the human intake and metabolism of *Citrus* flavonoids. Long-term clinical trials on the intake, metabolism and cytotoxicity of *Citrus* flavonoids are required before a diet recommendation for an increase intake for the prevention or treatment of human cancers.

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