Luteolin, a Flavonoid with Potential for Cancer Prevention and Therapy

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Abstract: Luteolin, 3',4',5,7-tetrahydroxyflavone, is a common flavonoid that exists in many types of plants including fruits, vegetables, and medicinal herbs. Plants rich in luteolin have been used in Chinese traditional medicine for treating various diseases such as hypertension, inflammatory disorders, and cancer. Having multiple biological effects such as anti-inflammation, anti-allergy and anticancer, luteolin functions as either an antioxidant or a pro-oxidant biochemically. The biological effects of luteolin could be functionally related to each other. For instance, the anti-inflammatory activity may be linked to its anticancer property. Luteolin's anticancer property is associated with the induction of apoptosis, and inhibition of cell proliferation, metastasis and angiogenesis. Furthermore, luteolin sensitizes cancer cells to therapeutic-induced cytotoxicity through suppressing cell survival pathways such as phosphatidylinositol 3'-kinase (PI3K)/Akt, nuclear factor kappa B (NF-κB), and X-linked inhibitor of apoptosis protein (XIAP), and stimulating apoptosis pathways including those that induce the tumor suppressor p53. These observations suggest that luteolin could be an anticancer agent for various cancers. Furthermore, recent epidemiological studies have attributed a cancer prevention property to luteolin. In this review, we summarize the progress of recent research on luteolin, with a particular focus on its anticancer role and molecular mechanisms underlying this property of luteolin.

Keywords: luteolin, cancer, therapy, prevention, ROS, apoptosis, carcinogenesis, flavonoid.

INTRODUCTION

Luteolin, 3',4',5,7-tetrahydroxyflavone, belongs to a group of naturally occurring compounds called flavonoids that are found widely in the plant kingdom. Flavonoids are polyphenols that play an important role in defending plant cells against microorganisms, insects, and UV irradiation [1]. Evidence from cell culture, animal, and human population studies have suggested that flavonoids are also beneficial to human and animal health. Because of their abundance in foods, e.g., vegetables, fruits, and medicinal herbs, flavonoids are common nutrients that are antioxidants, estrogenic regulators, and antimicrobial agents [2]. It has been noticed that flavonoids may be a cancer preventive [3, 4]. Flavonoids may block several points in the progression of carcinogenesis, including cell transformation, invasion, metastasis, and angiogenesis, through inhibiting kinases, reducing transcription factors, regulating cell cycle, and inducing apoptotic cell death [2].

Belonging to the flavone group of flavonoids, luteolin has a C6-C3-C6 structure and possesses two benzene rings (A, B), a third, oxygen-containing (C) ring, and a 2-3 carbon double bond. Luteolin also possesses hydroxyl groups at carbons 5, 7, 3', and 4' positions (Fig. **1**) [5]. The hydroxyl moieties and 2-3 double bond are important structure features in luteolin that are associated with its biochemical and biological activities [6]. As in other flavonoids, luteolin is often glycosylated in plants, and the glycoside is hydrolyzed to free luteolin during absorption [7]. Some portion of luteolin is converted to glucuronides when passing through the intestinal mucosa [8]. Luteolin is heat stable and losses due to cooking are relatively low [9].

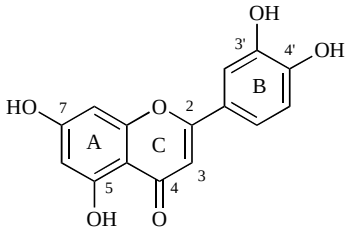

Fig. (1). Structure of luteolin.

Vegetables and fruits such as celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, apple skins, and chrysanthemum flowers are luteolin rich [4, 10-13]. Plants rich in luteolin have been used as Chinese traditional medicine for hypertension, inflammatory diseases, and cancer [1]. The pharmacological activities of luteolin could be functionally related to each other. For instance, the anti-inflammatory effect of luteolin also may be linked to its anticancer function. The anticancer property of luteolin is associated with inducing apoptosis, which involves redox regulation, DNA damage, and protein kinases in inhibiting proliferation of cancer cells and suppressing metastasis and angiogenesis. Furthermore, luteolin sensitizes a variety of cancer cells to therapeutically induced cytotoxicity through suppressing cell survival pathways and stimulating apoptosis pathways. Notably, luteolin is blood-brain barrier permeable, rendering it applicable to the therapy of central nerve system diseases, including brain cancer [14]. Furthermore, recent studies have attributed a cancer prevention potential to luteolin. In this review, we summarize recent progress in luteolin researches.

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1568-0096/08 $55.00+.00

Particularly, we focus on the roles and molecular mechanisms underlying luteolin's anticancer property.

REDOX MODULATION ACTIVITY

Antioxidant Activity

Most flavonoids, including luteolin, are regarded as antioxidants. Reactive oxygen species (ROS) refers to a diverse group of reactive, short-lived, oxygen-containing species, such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$), and lipid peroxyl radical ($LOO^{\bullet}$). ROS serve as second messengers for cellular signaling [15]. However, excessive production of ROS results in oxidative stress and damage to DNA, lipids, and protein that is involved in cancer as well as cardiovascular and neurodegenerative diseases. Luteolin was found to inhibit ROS-induced damage of lipids, DNA, and protein [16, 17].

Multiple mechanisms may underlie luteolin's antioxidant effect. First, luteolin functions as a ROS scavenger through its own oxidation [18]. Luteolin possesses the structures essential to flavonoid's antioxidant activity: 3', 4' hydroxylation, the presence of a double bond between carbons 2 and 3, and a carbonyl group on carbon 4 [18]. The hydrogen atom from an aromatic hydroxyl group can be donated to free radicals. As an aromatic compound, luteolin can support unpaired electrons around the M-electron system [17, 18]. Direct evidence showing luteolin as a ROS scavenger was obtained in cell-free systems [19]. Second, luteolin inhibits ROS-generating oxidases. For example, luteolin suppresses $O_2^{\bullet-}$ formation by inhibiting xanthine oxidase activity [20]. However, it is unclear in mammalian cells whether luteolin affects ROS generation in the mitochondria, the main ROS generation site, although it interferes with the mitochondrial electron transportation chain in parasite (leishmanial) cells [21]. Third, luteolin may exert its antioxidant effect by protecting or enhancing endogenous antioxidants such as glutathione-S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) [5, 22, 23]. Fourth, luteolin may directly inhibit the enzymes that catalyze oxidation of the cellular components. For example, luteolin suppresses lipoxygenase, cyclooxygenase, and ascorbic acid-stimulated malonaldehyde formation in liver lipids [16]. Lastly, luteolin may chelate transition metal ions responsible for the generation of ROS and therefore inhibit lipooxygenase reaction, or suppress nontransition metal-dependent oxidation [5, 17]. It should be noted that concordant antioxidant mechanisms of luteolin may occur *in vivo*. For example, inhibition of LPS-induced $^{\bullet}OH$ production in macrophages by luteolin may be through scavenging $O_2^{\bullet-}$, inhibiting xanthine oxidase activity, or a combination of both [24].

Pro-Oxidant Activity

Although the ability of flavonoids to protect cells from oxidative stress has been well-documented, there is increasing evidence for their pro-oxidant property [25, 26]. The pro-oxidant activity of flavonoids may be related to their ability to undergo autoxidation catalyzed by transition metals to produce superoxide anions [27]. In other reports, however, it was observed that the phenol rings of flavonoids are metabolized by peroxidase to form pro-oxidant phenoxyl radicals, which are sufficiently reactive to co-oxidize glutathione (GSH) or nicotinamide-adenine hydrogen (NADH) accompanied by extensive oxygen uptake and ROS formation [28]. The structure-activity relationship study on pro-oxidant cytotoxicity of flavonoids shows that flavonoids with a phenol ring are generally more bioactive than the catechol ring-containing ones [28]. Cytotoxicity induced by flavonoids is correlated with their electrochemical oxidation susceptibility and lipophilicity [29]. Luteolin has been shown to induce ROS in untransformed and cancer cells [30, 31]. In lung cancer cells, luteolin induced accumulation of $O_2^{\bullet-}$ while it reduced H_2O_2 concentration. Although a suppression of manganese superoxide dismutase (MnSOD) activity, which converts $O_2^{\bullet-}$ to H_2O_2, was observed, it remains to be determined whether other mechanisms underlie luteolin-induced pro-oxidation [31].

How the anti- or pro-oxidant effects of luteolin ensue has not been well determined. It is believed that flavonoids could behave as antioxidants or pro-oxidants, depending on the concentration and the source of the free radicals [32]. Also, the context and microenvironment of the cell may be important determinants of the outcome of luteolin-induced effects on cellular redox status. For example, the antioxidant activity of luteolin is dependent on Cu, V, and Cd ions in the cells. Changes in the Fe ion concentrations dramatically impact the effect of luteolin's redox-regulating activities. With low Fe ion concentrations (< 50 μM), luteolin behaves as an antioxidant while high Fe concentrations (>100 μM) induce luteolin's pro-oxidative effect [33].

Understanding whether and how luteolin's redox regulation activity is involved in its cellular effects is key to evaluating its potential as an anticancer agent, a cardioprotectant, or an inhibitor of neurodegeneration [34]. Because oxidative stresses are closely related to mutagenesis and carcinogenesis, luteolin, as an antioxidant, may act as a chemopreventive agent to protect cells from various forms of oxidant stresses and thus prevent cancer development. On the other hand, the pro-oxidant properties of luteolin may be involved in its ability to induce tumor cell apoptosis, which is achieved partly through direct oxidative damage of DNA, RNA, and/or protein in the cells [30, 35]. Interference of cellular signaling by ROS may also contribute to luteolin-induced apoptosis in cancer cells. We found that luteolin-induced oxidative stress causes suppression of the NF-κB pathway while it triggers JNK activation, which potentiates TNF-induced cytotoxicity in lung cancer cells [31]. It was suggested that the antioxidant activity of luteolin is associated with apoptosis in lung cancer cell line CH27. However, the induction of SOD-1 and -2 proteins by luteolin is moderate, and no causative relationship between the induction of SOD proteins and suppression of ROS or apoptosis was established [22]. Thus, the anti- and pro-oxidant roles of luteolin in cytotoxicity need to be further investigated.

ESTROGENIC AND ANTI-ESTROGENIC ACTIVITY

Estrogens are hormones involved in the proliferation and differentiation of their target cells. In response to estrogens, the estrogen receptor (ER) is activated to stimulate DNA synthesis and cell proliferation [36]. Flavonoids are naturally

occurring phytoestrogens because they can bind to ERs and activate their signaling pathways [37-39]. Because luteolin possesses potent estrogenic activity at low concentrations, it could be a useful agent for hormone replacement therapy [40].

However, there are also reports showing anti-estrogenic effects of luteolin [38]. The mechanism behind this apparently contradictory effect may be attributed to its relative low estrogenic activity when it binds to ERs. Flavonoids bind and activate ERs when estrogen is deficient. However, due to their relative weak estrogenic activity, which is 10^3- to 10^5-fold lower than 17-β-estradialluteolin, they may function as anti-estrogenic agents through competition with estrogens for binding to ERs [40, 41]. Another mechanism of luteolin's anti-estrogenic activity is that it inhibits aromatase whose function is to aromatize androgens and produce estrogens [42]. Additionally, luteolin reduces the ER expression level through inhibiting transcription of the ER gene or potentiating degradation of the ER protein [43, 44]. Finally, some alternative signaling mechanisms unrelated to ERs could also be involved [37]. Although the interaction of estrogen agonists and antagonists with the ER is a primary event in estrogen action, mammalian cells contain a second binding site (type II site) for estrogen to control cell growth, which resides in endogenous proteins such as histone [45]. Luteolin was found to bind to nuclear type II sites irreversibly and to compete for estradiol binding to these sites [46].

The etiology of breast, prostate, ovarian, and endometrial cancers is associated with estrogen activity. Thus, consumption of luteolin in diet may reduce risk of these cancers through regulation of estrogen-induced cellular effects. Indeed, luteolin, as well as other flavonoids, is able to inhibit DNA synthesis and proliferation in mammary epithelial cells and breast cancer cells induced by estrogens, both *in vitro* and *in vivo* [38, 47]. Suppressing estrogen-induced cancer cell proliferation may contribute to luteolin's therapeutic and preventive activities against estrogen-associated cancer.

ANTI-INFLAMMATION

Inflammation is one of the body's defense mechanisms that guard against infection and help heal injury. However, chronic inflammation may result in harmful diseases such as arthritis, chronic obstructive pulmonary disease, and cancer [48-50]. During inflammation macrophages are activated by various molecules, including cytokines from the host and toxins from the pathogens. Lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacteria, is a common endotoxin and inflammation trigger. The activated macrophages vigorously produce inflammatory molecules such as tumor necrosis factor α (TNFα), interleukins (ILs), and free radicals (ROS and reactive nitrogen species, RNS), leading to recruitment of inflammatory cells, such as neutrophils and lymphocytes, to the infection site and clearance of the pathogens [48, 50]. Persistent production of these molecules during chronic inflammation can result in diseases such as cancer. Luteolin exerts its anti-inflammatory effect through suppressing the production of these cytokines and their signal transduction pathways [51-53]. Experiments with animals show that luteolin suppresses LPS or bacteria-induced inflammation *in vivo* [54, 55]. LPS-induced-high mortality was effectively alleviated by luteolin, which is associated with reduction of LPS-stimulated TNFα release in serum and intercellular adhesion molecule-1 (ICAM-1) expression in the liver [54]. Luteolin was found to suppress inflammation in lung tissue that was caused by *Chlamydia pneumoniae* [55].

In vitro experiments provided more direct evidence of luteolin's anti-inflammatory effect. Pretreating murine macrophages (RAW 264.7) with luteolin inhibited LPS-stimulated TNFα and IL-6 release, which was associated with blockage of LPS-induced activation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) family members ERK, p38, and JNK [51, 52, 56, 57]. NF-κB and MAPK are two major pathways that are involved in macrophage activation and in responses of tissue epithelial and stromal cells to inflammation mediators such as TNFα and ILs [58]. Suppression of these pathways by luteolin underlies the main mechanism of its inhibitory effect on both acute and chronic inflammation. The suppression of inflammatory cytokine-induced signaling is at least partly on the level of the receptor, because accumulation of lipid rafts, which is the critical step for receptor signaling, was blocked by luteolin [53].

NF-κB can be activated by both the primary (LPS) and secondary (TNFα and IL-1) inflammatory stimulators. As a heterodimer typically consisting of RelA (p65)/p50, NF-κB is retained in the cytoplasm as an inactive form by association with IκB proteins. Through binding to Toll-like receptor 4 (TLR-4), LPS activates the IκB kinase (IKK), which in turn phosphorylates IκB to trigger its rapid degradation. This allows NF-κB to migrate into the nucleus and activate its targets, including a number of genes with anti-apoptotic properties and cytokines such as TNFα and IL-1 [59]. A positive feedback loop for NF-κB activation is established by these cytokines through binding to their cognate receptors. The NF-κB pathways activated by LPS and the inflammatory cytokines converge at IKK activation [59]. Luteolin can effectively block the NF-κB pathway and interfere with the functions of the primary (LPS) and secondary (TNFα and IL-1) inflammatory stimulators through inhibiting IKK activation and IκB degradation [51, 56, 60]. However, it remains to be determined whether luteolin directly inhibits IKK activity or blocks the upstream steps in the IKK activation pathway such as the formation of the receptor signaling complex. On the other hand, the mechanism by which luteolin suppresses MAPK, which is awaiting the dissection of the MAPKKK-MAPKK-MAPK cascade for each MAPK activation, is less well understood. It is unlikely that luteolin suppresses the binding of TNFα and IL-1 to their respective receptors because luteolin selectively suppresses each MAPK in macrophages [57].

Based on the observations that some flavonoids with strong antioxidant activities are completely ineffective in suppressing LPS-stimulated TNFα production, it is assumed that the inhibitory action of flavonoids on proinflammatory cytokine production is not directly associated with their antioxidant properties [61]. However, because luteolin is able to scavenge ROS directly and to suppress the LPS-activated nitric oxide production in activated macrophages, the antioxidant activity of luteolin at least in part contributes to luteolin's anti-inflammatory effect [62, 63]. Because inflamma-

tion and its involved signaling pathways are strongly associated with carcinogenesis [64, 65], luteolin's anti-inflammation role may contribute to cancer prevention.

ANTI-CANCER ACTIVITIES

Carcinogenesis is a long-lasting and multi-stage process that results from clonal expansion of mutated cells. A typical carcinogenic process can be divided into three stages: initiation, promotion, and progression. During initiation, a potential carcinogen (pro-mutagen) is converted to a mutagen by enzymes such as cytochrome P450. The mutagen then reacts with DNA to induce irreversible genetic alteration including mutations, transversions, transitions, and/or small deletions in DNA. During the promotion stage, alterations in genome expression occur to favor cell growth and proliferation. During the progression stage tumorigenicity is established and becomes irreversible; it is characterized by karyotypic instability and malignant growth in an uncontrolled manner [66]. The transformed cells acquire a number of characteristic alterations, including the capacity to proliferate in an exogenous growth-promoting signal-independent manner, to invade surrounding tissues and metastasize to distant sites. In addition, cancer cells elicit an angiogenic response, evade mechanisms that limit cell proliferation (such as apoptosis and senescence), and elude immune surveillance [67]. These properties of cancer cells are reflected by alterations in the cellular signaling pathways that control cell proliferation, motility, and survival in normal cells [67]. Luteolin is able to interfere with almost all of the characteristics of cancer cells, mainly through the following mechanisms [68]. The main potential molecular targets for luteolin's anticancer activity are summarized in Table **1**.

Preventing Carcinogen Metabolic Activation

In earlier reports, luteolin was found to inhibit the metabolism of carcinogens that generates active mutagens in liver microsomes [69, 70]. Recently, it was determined that luteolin potently inhibits human cytochrome P450 (CYP) 1 family enzymes such as CYP1A1, CYP1A2, and CYP1B1, thereby suppressing the mutagenic activation of carcinogens [71]. Suppressing these enzymes reduces the generation of active mutagens such as benzo[a]pyrene diol epoxide, a metabolite of the tabacco-specific carcinogen benzo[a] pyrene carcinogenesis [72].

Inhibiting Cancer Cell Proliferation

Unrestricted proliferation, which is often due to lose of cell cycle control, allows cancer cells to outgrow and form tumors. Like many other flavonoids, luteolin is able to inhibit the proliferation of cancer cells derived from nearly all types of cancers, mainly through regulating the cell cycle [38, 73-75].

In eukaryotic cells, proliferation proceeds through DNA replication followed by division of the nucleus and separation of the cytoplasm to yield daughter cells. The sequential process, called cell cycle, consists of four distinct phases, G1, S, G2, and M [76]. Cell cycle progression is timely regulated by cyclin-dependent kinases (CDKs) and their cyclin subunits at the two checkpoints, G1/S and G2/M [76]. The G1/S checkpoint is regulated by CDK4-cyclin D, CDK6-cyclin D, and CDK2-cyclin E. When associated with cyclin A, CDK2 controls the S-phase, while the G2/M transition is regulated by CDK1 in combination with cyclins A and B [76]. CDK activity is negatively controlled by two groups of CDK inhibitors (CKI), INK4 and CIP/KIP families. The INK4 family members inhibit CDK4 and CDK6; while the CIP/KIP family, consisting of p21cip1/waf1, p27kip1, and p57kip2, inhibits a broad range of CDKs [76].

Inhibiting Cell Cycle Progression

Flavonoids have been found to inhibit the proliferation of many cancer cells by arresting cell cycle progression either at the G1/S or G2/M checkpoint [77, 78]. Luteolin is able to arrest the cell cycle during the G1 phase in human gastric and prostate cancer, and in melanoma cells [79-81]. The G1 cell cycle arrest induced by luteolin is associated with inhibition of the CDK2 activity in melanoma OCM-1 and colorec-

Table 1. Potential Molecular Targets for Luteolin's Anticancer Activity*

Luteolin's effects	Processes Involved in Carcinogenesis					
	Carcinogen activation	**Cell Proliferation**	**Cell survival signaling**	**Apoptosis**	**Angiogenesis**	**Metastasis**
Activation		p27/kip1 [81,82] p21/waf1 [81,82] p53 [81,84,85]		DR5 [105] Caspases [105] Fas [106] Bax [108,110] p53 [107,108] JNK [103,104]		
Inhibition	CYP1A1 [71] CYP1A2 [71] CYP1B1 [71]	PKC [96] MAPKs [88,89] PI3K/Akt [88,90] CDK2 [81,82] NF-κB [97,98] PDGF [91] ER [38,44,75] IGF-IR [90]	IGF-IR [90] PDGF [91] PI3k/Akt [88,90] EGFR [89] ER [38, 40-44,93] PKC [113] NF-κB [31,109] MAPKs [88,89]	DNA topoisomerases [85,87] XIAP [113] Bcl-XL [114] Fatty acid synthase [115]	VEGF [121] VEGFR [73,120] MMPs [126] NF-κB [126] HIF-1α [121, 122] Hyaluronidase [124] PI3k/Akt [120] EGFR [102,132]	TNFα [51,56,129,130] IL-6 [131] MMP-1 [131] FAK [133] ERK [134,135] NF-κB [31,109] PI3k/Akt [134,135]

*Some factors are involved in multiple processes. For details please refer cited literature.

tal cancer HT-29 cells. This arrest is achieved by up-regulation of the CDK inhibitors p27/kip1 and p21/waf1, or direct inhibition on the CDK2 activity [81, 82]. Luteolin arrests mouse cancer cell tsFT210 at the G2/M checkpoint [83]. DNA damage-activated tumor suppressor protein p53 is involved in both the G1/S and G2/M transition regulation [84, 85]. Luteolin can bind and suppress DNA topoisomerases I and II, enzymes essential for repairing damaged DNA, and intercalates directly with the substrate DNA to cause DNA double-strand breaks [85-87]. This action of luteolin induces cell cycle arrest though p53-mediated expression of p21/waf1[81].

Suppressing Growth Factor Receptor-Mediated Cell Proliferation Signaling

Growth factors promote DNA synthesis and cell cycle progression through binding to their respective receptors. Common growth factors include epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF). TNFα can also stimulate cancer cell proliferation through NF-κB. The inhibitory effect of luteolin on cancer cell proliferation is partly achieved through blocking the proliferation signaling pathways induced by these factors.

EGF receptor (EGFR) is a typical receptor protein tyrosine kinase (PTK) that mediates cell growth and proliferation. When activated by its ligands, EGFR is phosphorylated to mediate activation of downstream signaling pathways, including MAPK and PI3K/Akt [88]. Luteolin was found to inhibit the proliferation of pancreatic and prostate cancer and human epidermoid carcinoma cells, which is closely associated with the inhibition of the PTK activity and autophosphorylation of EGFR, transphosphorylation of EGFR downstream effector protein enolase, and activation of MAPK/ERK [89].

Luteolin is able to inhibit IGF-1-induced activation of IGF-1R and Akt, and phosphorylation of the Akt targets p70S6K1, GSK-3β, and FKHR/FKHRL1. This inhibition is associated with suppressed expression of cyclin D1, and increased expression of p21/waf1 and proliferation in prostate cancer cells *in vitro* [90]. Luteolin also suppressed prostate tumor growth *in vivo* through suppressing IGF-1R/Akt signaling [90]. Similarly, luteolin inhibits PDGF-induced proliferation by inhibiting PDGF receptor phosphorylation in vascular smooth muscle cells [91]. As a consequence, luteolin significantly inhibits PDGF-induced ERK, PI3K/Akt and phospholipase C (PLC)-γ1 activation, and *c-fos* gene expression. These results suggest that the inhibitory effect of luteolin on the PDGF-induced proliferation may be mediated by blocking phosphorylation of the PDGF receptor [91]. As PDGF stimulates cancer cell proliferation [92], it remains to be determined whether luteolin can block PDGF-induced signaling to suppress cancer cell proliferation.

As discussed above, ER induces proliferation in several types of cancer cells [5]. Luteolin suppresses proliferation of prostate and breast cancer cells in both an androgen-dependent and -independent manner, suggesting that luteolin's anti-estrogen activity may at least partly contribute to its anti-proliferation effect [38, 44, 75]. Similar observations were also made in thyroid carcinoma cell lines bearing the ER [93]. Further experiments suppressing the expression and function of the ER are needed to confirm the role of ER-mediated signaling in luteolin-induced anti-proliferation in ER-responsive cancer cells.

In addition to affecting the receptors, luteolin may directly target the downstream pathways that are involved in cell proliferation. For example, protein kinase C, a family of serine-threonine protein kinases that regulates growth factor response and cell proliferation, differentiation and apoptosis [94, 95], can be inhibited in a concentration-dependent manner by luteolin in both cell-free systems and in intact cells [96].

Taken together, the above reports suggest that luteolin suppresses cell proliferation signaling on distinct components of the growth factor receptor signaling pathways. In addition, carcinogens activate cell survival pathways such as NF-κB and MAPK during the course of carcinogenesis; these pathways could be additional targets for flavonoids, including luteolin, in anti-carcinogenesis [97, 98].

Eliminating Transformed Cells by Induction of Apoptosis

Accumulating evidence shows that uncontrolled proliferation of mutated cells due to lack of programmed cell death or apoptosis is closely associated with tumorigenesis [99]. Cancer cells' resistance to apoptosis is acquired through a variety of biochemical changes that also contribute to cells' reduced responsiveness to anticancer therapy. Apoptosis is a tightly regulated cell death process that is critical for maintaining tissue homeostasis as well as preventing cancer development. Two apoptosis pathways, the death receptor pathway (extrinsic) and the mitochondrial (intrinsic) pathway, are established during evolution. The intrinsic pathway involves functional incapacitation of mitochondria by pro-apoptotic Bcl2 family members, including Bax, Bak, and Bik, that cause mitochondria potential loss and release cytochrome c to activate caspase 9, which in turn activates executor caspases (-3, -7) and destroys cellular proteins [100]. The extrinsic pathway is initiated by the binding of TNF family cytokines (TNFα, Fas and TNF-related apoptosis-inducing ligand, TRAIL) to their cognate death receptors, to activate caspase 8, which in turn activates downstream executor caspases [101].

Luteolin kills cancer cells by inducing apoptotic cell death in many types of cancer cells, including epidermoid carcinoma, leukemia, pancreatic tumor, and hepatoma [89, 102-104]. Although the mechanisms underlying luteolin-induced apoptosis are complex, they can be generalized as breaking the cell survival and death balance by either enhancing apoptosis or decreasing the survival signaling in cancer cells (Fig. **2**).

Activating the Apoptosis Pathway

Luteolin is potent to activate both the extrinsic and intrinsic apoptosis pathways. Direct increase in expression of the death receptor 5 (DR5), the functional receptor for TRAIL, has been demonstrated in cervical and prostate cancer cells, which is accompanied by activation of caspase-8, -10, -9 and -3, and cleavage of Bcl-2-interacting domain (BID). The increase of DR5 expression is likely through activated tran-

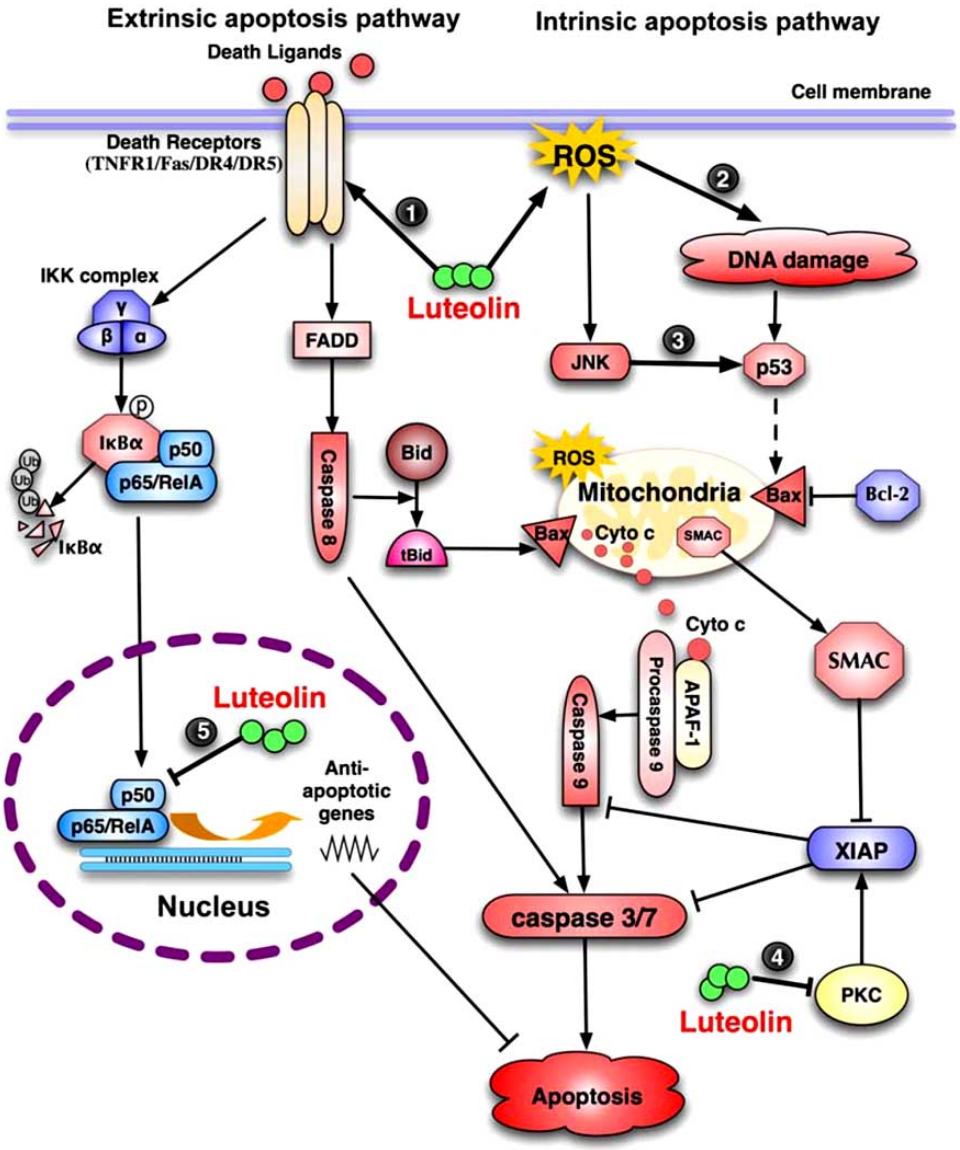

Fig. (2). Apoptosis pathways and the points targeted by luteolin. The extrinsic apoptosis pathway is mediated by death receptors, resulting in sequential activation of initiator caspase 8 and executor caspases 3 and 7. The intrinsic apoptosis pathway is initiated by lose of mitochondrial potential, which leads to release of cytochrome c. Cytochrome c binds to APAF-1 and procaspase 9, resulting in activation of initiator caspase 9 and downstream executor caspases. Cleavage of Bid by caspase 8 establishes a crosstalk between the extrinsic and intrinsic apoptosis pathways. Luteolin triggers apoptotic cell death through potentiation of both apoptosis pathways and suppression of cell survival pathways. The points targeted by luteolin in the apoptosis pathways are highlighted with numbers in filled circles.

scription of the *dr5* gene [105]. Interestingly, DR5 was not induced and no cytotoxicity was observed in luteolin-treated normal human peripheral blood mononuclear cells [105]. Luteolin was also found to enhance expression of Fas to induce apoptosis in human hepatoma cells through triggering the degradation of STAT3, a known negative regulator of *fas* transcription [106].

Luteolin also activates the intrinsic apoptosis pathway through inducing DNA damage and activating p53 [107, 108]. This is achieved by inhibiting DNA topoisomerases [85, 87]. Additionally, luteolin triggers sustained JNK activation that can promote the apoptosis pathway, presumably through modulation of BAD or p53 [31, 108-110]. The JNK-mediated p53 activation results in transcriptional expression of Bax that facilitates apoptosis [108, 110]. JNK activation causes the mitochondria translocation of Bax and Bak to initiate the intrinsic apoptosis pathway [103, 104].

Suppressing Cell Survival Signaling

On the other hand, luteolin suppresses cell survival pathways to decrease the threshold of apoptosis. As discussed above, luteolin inhibits survival pathways, such as PI3K/Akt, NF-κB, and MAPKs in cancer cells, which may mimic deprivation of growth factors that blocks the growth factor-triggered signaling pathways. Suppressing the death receptors-mediated cell survival pathway NF-κB augments apoptosis induced by their cognate ligands TNFα or TRAIL. TNFα plays a critical role in inflammation-associated carcinogenesis through NF-κB-mediated cell survival and proliferation [97, 111]. Blockage of NF-κB by luteolin shifts the

cell survival and death balance to the side of death [31, 109], converting TNFα from a tumor promoter to a tumor suppressor. TRAIL can promote proliferation and metastasis in TRAIL-resistant cancer cells *via* a mechanism involving NF-κB [112]; thus, suppressing NF-κB with luteolin can sensitize cancer cells to TRAIL-induced apoptosis and prevent the detrimental effect of TRAIL.

Luteolin also suppresses cell survival by inhibiting apoptosis inhibitors and anti-apoptotic Bcl2 family members. It was found that luteolin inhibits PKC activity, which results in a decrease in the protein level of XIAP by ubiquitination and proteasomal degradation of this anti-apoptotic protein. Reducing XIAP sensitizes cancer cells to TRAIL-induced apoptosis [113]. In addition to increasing Bax protein, luteolin decreases the Bcl-XL level in hepatocellular carcinoma cells, which elevates the Bax/Bcl-XL ratio and lowers the threshold for apoptosis [114]. Additionally, luteolin-induced apoptosis in prostate and breast cancer cells is associated with its ability to inhibit fatty acid synthase (FAS), a key lipogenic enzyme overexpressed in many human cancers [115]. Although presently the mechanism is unclear, inhibiting FAS causes apoptosis in cancer cells [116].

Anti-Angiogenesis

Due to lack of sufficient nutrition and oxygen, avascular tumors cannot grow beyond a diameter of 1-2 mm [117]. Angiogenesis, a process to generate new blood vessels, is critical for solid tumor growth and metastasis. Growing in a hypoxic microenvironment, tumor cells secret angiogenic factors such as vascular endothelial growth factor (VEGF) and matrix metalloproteases (MMP) to trigger angiogenesis [118]. Luteolin was found to be a potent angiogenesis inhibitor [119]. In a murine xenograft tumor model, luteolin inhibited tumor growth and angiogenesis in xenografted tumors [120].

Suppression of VEGF secretion and signaling induced by VEGF appears to be the main mechanism of luteolin-induced anti-angiogenesis. Transcription of the VEGF gene is enhanced by hypoxia-inducible factor-1α (HIF-1α) [121]. Luteolin may suppress VEGF expression by inhibiting HIF-1α through p53-mediated proteasomal degradation of this transcription factor [122]. Additionally, luteolin can suppress VEGF-induced signaling in endothelial cells [73, 120]. Luteolin effectively blocks activation of the VEGF receptor and its downstream PI3K/Akt and PI3K/p70S6 kinase pathways, which may directly contribute to luteolin-induced anti-angiogenesis, resulting in suppression of proliferation and survival of human umbilical vein endothelial cells [120].

Luteolin may also suppress angiogenesis by stabilizing hyaluronic acid, a neovascularization barrier. Hyaluronic acid is one of the most abundant constituents of the extracellular matrix that block neovacuole formation and extension [123]. Hyaluronidase catalyzes hyaluronic acid to break the barrier and to promote angiogenesis through the processed product. Oligosaccharides generated from hyaluronic acid bind to the CD44 receptor on the membranes of endothelial cells to trigger their proliferation, migration, and eventually angiogenesis. Luteolin has been found to be a potent inhibitor of hyaluronidase and maintain the neovascularization barrier [124].

Furthermore, tumor angiogenesis is dependent on the activity of MMPs, especially that of MMP-9, which renders MMP inhibitors a potential choice for blocking tumor angiogenesis [125]. Thus, luteolin's additional anti-angiogenesis mechanism may be *via* its suppression of MMPs. Indeed, luteolin is a potent MMP inhibitor that suppresses MMP expression through suppressing NF-κB or directly inhibiting MMP activity [126].

Anti-Metastasis

In addition to rapid and continuous division and proliferation, another important and unique feature of cancer cells is their ability to invade surrounding tissues and to migrate from their primary site to distal sites. This process, namely metastasis, contributes to over 90% of human cancer mortality [127]. The metastasis cascade is thought to consist of multiple steps: local invasion; intravasation into the systemic circulation; survival during transport, extravasation, and establishment of micrometastases in distant organs; and colonization of macroscopic metastases [128].

Although direct evidence showing luteolin suppresses cancer metastasis is not seen in literature, available results strongly suggest that luteolin has this function. First, luteolin suppresses production and secretion of cytokines such as TNFα and IL-6 that can stimulate cancer cell migration and metastasis [51, 56, 129, 130]. TNFα stimulates expression of molecules involved in cancer cell migration and metastasis such as intercellular adhesion molecule-1, which can be blocked by luteolin [52]. IL-6 is known to induce MMP-1 expression. Luteolin potently inhibits the production of IL-6 and IL-6-induced expression of MMP-1 [131]. Second, luteolin blocks critical signal transduction pathways for migration and metastasis in cancer cells. For example, activation of the EGFR is involved cell migration. By blocking the EGFR-signaling pathway, luteolin reduces cell invasion and metastasis [102, 132]. Luteolin blocks NF-κB [31, 109], which is critical for the expression of Twist and MMP expression. Twist is a transcription factor that is important for epithelial-mesenchymal transition to facilitate metastasis [128]. MMPs are involved in several stages of metastasis, including the escape of individual tumor cells from the primary tumor, their intravasation, extravasation, and establishment of tumor foci at secondary sites [125]. Focal adhesion kinase (FAK) activity in human carcinoma cells is associated with increased invasive potential; luteolin's inhibitory effect on FAK phosphorylation may contribute to suppressing FAK's cell invasion ability [133]. Finally, luteolin directly inhibits MMP or hyaluronidase enzyme activity to maintain the neovascularization barrier [124, 126], which may also contribute to suppressing cancer cell metastasis. *In vitro* studies have shown that luteolin potently inhibits migration and invasion of cancer cells through blocking the MAPK/ERKs and PI3K-Akt pathways [134, 135]. Experiments with cancer metastasis animal models are needed to verify luteolin's anti-metastasis activity.

LUTEOLIN AS AN ANTICANCER OR CHEMOPREVENTION AGENT

As discussed above, luteolin induces apoptotic cell death in a variety of cancers [103, 104, 136, 137], inhibits cancer

cell proliferation [82, 90, 138], and suppresses tumor angiogenesis [120]. Thus, luteolin is expected to be a putative anticancer therapeutic. Supporting the *in vitro* results, *in vivo* experiments in nude mice with xenografted tumors showed that luteolin suppressed growth of tumors formed from human skin carcinoma, hepatoma, and human ovarian cancer cells [106, 120, 137] or mouse Lewis lung carcinoma [139] in a dosage-dependent manner. Interestingly, in a 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis in Wistar rat model, luteolin inhibited the incidence rate of tumors and decreased tumor volume significantly without changing the total body weight of the animals. Long-term treatment did not show any apparent toxicity in rats (30mg/kg, p.o. for 20 days)[140]. Consistently, luteolin induces marginal cytotoxicity in normal cells [105, 141]. These results imply that luteolin is relatively safe when used as an anticancer agent.

Combination therapy with distinct anticancer drugs can improve the therapeutic value of the combined agents by allowing the use of lower, subtoxic doses to achieve more effective cancer cell killing. Luteolin has been tested with other anticancer drugs for its anticancer cell properties, and has sensitized different drug-induced cytotoxicity in a variety of cancer cells. The drugs tested include cisplatin [108], TRAIL [105, 113], TNFα [31, 109], and the mTOR inhibitor rapamycin [137]. Although the mechanism of this sensitization vary in different cancer cells or with different drugs, it is generally thought to be through suppressing cell survival signals in cancer cells or activating apoptosis pathways. Cancer cells often have constitutively activated cell survival pathways such as NF-κB and Akt. Cancer therapeutics also activate these pathways, dampening their cancer cell-killing activities [142, 143]. Thus, luteolin's suppression of the constitutive or drug-induced cell survival pathways contributes to the sensitized anticancer activity. Additionally, luteolin is also capable of promoting apoptotic pathways. For instance, luteolin-induced upregulation of the TRAIL receptor DR5 contributes to sensitizing not only TRAIL-induced, but also other chemotherapeutic-induced cytotoxicity [144].

Thus, data from previous studies suggest luteolin is a promising agent for anticancer therapy. More preclinical work is needed for establishing the efficacy and safety of luteolin alone or in combination with other therapeutics before conducting clinical trials. Because extracts from fruits such as black raspberries, apples and grapes exerts anticancer activities that are associated with suppressing of cell survival and potentiation of apoptosis pathways, it is interesting to determine if luteolin or other flavonoids contributes to the anticancer activity of these fruits [145-149].

Based on the observations that luteolin is able to interfere with almost all the aspects of carcinogenesis, and it is relatively safe for animals and humans, it is assumed to be a potential chemopreventive agent against cancer through blocking cell transformation, suppressing tumor growth, and killing tumor cells. Using luteolin to suppress chronic inflammation can potentially prevent inflammation-associated carcinogenesis.

In a 20-methycholanyrene-induced fibrosarcoma model using Swiss albino mice, luteolin administered in diet significantly suppressed tumor incidences, which are associated with reduction in lipid peroxides and cytochrome P450, increased activity of GST, and suppressed DNA synthesis [150]. In a murine two-stage skin carcinogenesis model, topical application of luteolin prior to 12-tetradecanoylphorbol 13-acetate (TPA) treatment in DMBA-initiated mouse skin resulted in a significant reduction in tumor incidence and multiplicity, which is associated with inhibiting the inflammatory response and scavenging reactive oxygen radicals [151, 152]. In a colon carcinogenesis model induced by 1, 2-dimethyl hydrazine (DMH), luteolin (0.1, 0.2, or 0.3 mg/kg body weight/daily p.o.) significantly reduced colon cancer incidence when it was administered at either the initiation or post-initiation stages [153]. The results demonstrate that luteolin exerts chemopreventive and anticarcinogenic effects, in association with its antiperoxidative and antioxidant effects, against colon cancer [153].

Epidemiological studies suggest that dietary intake of flavonoids is inversely associated with risk of lung, prostate, stomach, and breast cancer in humans [4, 154, 155]. However, there are few epidemiological reports designed to study the role of luteolin in cancer prevention. A recent population study on the association between intake of dietary flavonoids and incidence of epithelial ovarian cancer among 66,940 women showed a significant (34%) decrease in cancer incidence for the highest versus lowest luteolin intake (RR = 0.66, 95% CI = 0.49–0.91; p-trend = 0.01) [11]. The data suggest that dietary intake of luteolin may reduce ovarian cancer risk, although additional prospective studies are needed [11]. Dietary intake of flavonols and flavones was found to be inversely associated with the risk of lung cancer [3, 156]. However, because of many confounding factors, luetolin's preventive potential for lung cancer still remains unclear [156, 157]. It should be noted that mixed bioactive compounds, such as different flavonoids that exist in foods, may impact each others' biological effects. Lifestyle differences of the subjects in a study may interfere with the results. Furthermore, variations in epidemiological studies, including differences in questionnaire design, databases for flavonoid content in foods, and methods for data analysis, may substantially vary the outcomes of different studies. Thus, caution should be exercised when interpreting epidemiological study results [4]. Nevertheless, further prospective animal and human studies are warranted to verify luteolin's cancer prevention properties.

CONCLUSIONS AND PERSPECTIVES

Documented results suggest that luteolin has a variety of beneficial properties, including those as an anti-inflammatory and anticancer agent. The mechanisms underlying these properties have not been fully understood but are attributed partly to luteolin's redox- and estrogen-regulating properties. It is interesting and important to determine the mechanism for luteolin's selective cytotoxicity in cancerous but not normal cells. It is apparent that distinct mechanisms for modulating cellular signaling pathways exist in normal cells and in malignant cancer cells. For example, luteolin suppresses JNK in macrophages while it activates this kinase in cancer cells [31, 57, 109]. Also, luteolin suppresses NF-κB through inhibiting IKK activation during inflammation in epithelial cells and macrophages [51, 56, 158]. However, in

cancer cells suppression of NF-κB by luteolin is apparently a nuclear event [31, 109]. It remains to be determined whether the distinct mechanisms are due to differences in cell contexts. Because luteolin inhibits NF-κB in lung cancer cells and is associated with its pro-oxidant effect [31], it will be interesting to determine if the distinct mechanisms in NF-κB suppression are dependent on the redox status of the cell or the redox-regulating function of luteolin. Understanding the mechanisms will undoubtedly facilitate the use of luteolin in cancer prevention and therapy. Finally, although it is relatively safe, luteolin (2% in chow diet) was found to worsen chemically induced colitis in mice [159]. Further studies are needed to address the safety issues of luteolin with doses effective for cancer prevention and therapy in humans.

ACKNOWLEDGEMENTS

The authors thank Dr. Guodong Lu for helping figure preparation and Vicki Fisher for editing the manuscript. This study is partly supported by grants from the National Cancer Institute (R03CA125796 to Y.L.), National Medical Research Council (NMRC) and Biomedical Research Council (BMRC), Singapore (to H-M.S) and National Natural Science Foundation of China (30772539, to X.W.).

ABBREVIATIONS

BID = Bcl-2-interacting domain

CAT = catalase

CDK = cyclin-dependent kinase

CKI = CDK inhibitors

CYP = cytochrome P450

DMBA = 7,12-dimethylbenz[a]anthracene

DMH = 1, 2-dimethyl hydrazine

DR5 = death receptor 5

EGF = epidermal growth factor

EGFR = epidermal growth factor receptor

ER = estrogen receptor

FAK = focal adhesion kinase

FGF = fibroblast growth factor

GR = glutathione reductase

GSH = glutathione

GST = glutathione-S-transferase

H_2O_2 = hydrogen peroxide

HIF-1α = hypoxia-inducible factor-1α

IGF = insulin-like growth factor

ICAM-1 = intercellular adhesion molecule-1

IKK = IκB kinase

IL = interleukin

LPS = lipopolysaccharide

$LOO^{\bullet}$ = lipid peroxyl radical

MAPK = mitogen-activated protein kinase

MMP = matrix metalloproteases

MnSOD = manganese superoxide dismutase

NADH = nicotinamide-adenine hydrogen

NF-κB = nuclear factor kappa B

$O_2^{\bullet-}$ = superoxide

$^1O_2$ = singlet oxygen

$^{\bullet}OH$ = hydroxyl radical ($^{\bullet}OH$)

PDGF = platelet-derived growth factor

PI3K = phosphatidylinositol 3'-kinase

PKC = protein kinase C

PTK = protein tyrosine kinase

ROS = Reactive oxygen species

RNS = reactive nitrogen species

$^1SO_2$ = singlet oxygen

SOD = superoxide dismutase

TLR-4 = Toll-like receptor-4

TNFα = tumor necrosis factor alpha

TRAIL = TNF-related apoptosis-inducing ligand

TPA = 12-tetradecanoylphorbol 13-acetate

VEGF = vascular endothelial growth factor

XIAP = X-linked inhibitor of apoptosis protein

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Received: April 21, 2008 **Revised: July 29, 2008** **Accepted: July 30, 2008**