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Anti-cancer Effect and Underlying Mechanism(s) of Kaempferol, a Phytoestrogen, on the Regulation of Apoptosis in Diverse Cancer Cell Models

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Phytoestrogens exist in edible compounds commonly found in fruits or plants. For long times, phytoestrogens have been used for therapeutic treatments against human diseases, and they can be promising ingredients for future pharmacological industries. Kaempferol is a yellow compound found in grapes, broccoli and yellow fruits, which is one of flavonoid as phytoestrogens. Kaempferol has been suggested to have an antioxidant and anti-inflammatory effect. In past decades, many studies have been performed to examine anti-toxicological role(s) of kaempferol against human cancers. It has been shown that kaempferol may be involved in the regulations of cell cycle, metastasis, angiogenesis and apoptosis in various cancer cell types. Among them, there have been a few of the studies to examine a relationship between kaempferol and apoptosis. Thus, in this review, we highlight the effect(s) of kaempferol on the regulation of apoptosis in diverse cancer cell models. This could be a forecast in regard to use of kaempferol as promising treatment against human diseases.

Key words**:** Phytoestrogen, Kaempferol, Apoptosis, Cancer models, Estrogen receptors

INTRODUCTION

Typically, cells are maintained through cell division, growth and arrest. However, abnormal cell regulatory processes could lead to excessive growth of cells and become cancers (1). Cancer growths unexpectedly invade into normal tissue or organ through more than required unlimited proliferation. And then they metastasize into many organs and interrupt normal cell function. In cancer progression, DNA repair system is disrupted, and inflammation, angiogenesis and apoptosis are altered in cancer cells (2). Main treatments to cure cancers include surgical removal, radiation treatment or chemotherapy, while other hormonal or biological gene therapy have been proposed for cancer treatments as well (3).

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As a substitute, phytoestrogen as a natural substance has been proposed for cancer patient treatments. Phytoestrogens derived from fruits and vegetables reduced general risk of diverse human cancers (4). In particular, some endocrine cancers may be influenced by hormones, such as estrogen, androgen or cortisol etc. Because of the similar molecular structures between phytoestrogens and endogenous steroid hormones, these phytoestrogens may have similar functions in our body (5). Thus, phytoestrogens are used as hormone replacement therapy (HRT) for prolonged therapy, for instance, the prevention of breast cancer, postmenopause, and osteoporosis (6). Thus, phytoestrogens may be employed for potential therapy as proposed by substantial evidence.

In past decade, many studies have been done to clarify relationship between phytoestrogens and cancers for an alternative treatment. Risk of heart disease, osteoporosis and various cancers could be reduced by high consume of legumes and soy products (7). *In vitro* and *in vivo* studies suggest that isoflavonoid may inhibit cancer cell growth by competing estradiol to type 2 estrogen binding sites (8). Genistein derived from isoflavones could inhibit cancer progression induced by 17beta-estradiol or various endocrine disrupting chemicals by regulating cell cycle related or insulin-growth factor-1 (IGF-1) signaling pathway in BG-1 ovarian cancer cells expressing estrogen receptor (ER)s (9,10). However, all of phytoestrogens may not be applicable since detailed underlying mechanism needs to be uncovered in the inhibition of cancer cell growth by phytoestrogens (8).

Kaempferol is a yellow compound that belongs to the flavonoid (11). This is mostly included in fruits such as apple, grape and tomato and in plants such as green tea, pine, *angelica decursiva* and ginkgo leaf (12-14). Kaempferol has been shown to have an antioxidant activity and anti-inflammatory function such as flavonoids (11). Kaempferol may also reduce the risk of cardiovascular and neuroinflammatory diseases (15). Taken together, this may have a therapeutic potential for cancer prevention. There are substantial studies to reveal the underlying mechanism(s) of kaempferol on the regulation of apoptosis (16-18). Among them, we will highlight the detailed effects of kaempferol on the regulation of apoptosis in this review.

Phytoestrogens. From 50 years ago, phytoestrogen attention comes from Western Australia, inspired by sheep which changed reproduction ability when fed red clover (19). Dietary intake of phytoestrogens, i.e., tobu and bean products, is quite different and diverse by each country and culture, while the uptake ratio of phytoestrogen-rich diet is much higher in Asian counties. Epidemiological studies suggest that prostate, breast and colorectal cancers called Western disease have occurred lower in Asian counties than Westerns (20). By the same reason, flushing of postmenopausal women is lower clinically in Asian counties (21). Estrogen deficiency due to postmenopausal affect occurrence of chronic diseases associated with aging (22). Phytoestrogen supplement could reduce or mitigate the number of symptoms that came from menopause. Isoflavones have a protective effect on bones in women, particularly in lumbar spine (23). They could act on cells or tissues by competitively binding to ERs with endogenous estrogens during developmental and reproductive stages. Actual research shows that phytoestrogens have been shown to have diverse effects depending on their concentrations (24). For example, kaempferol has been demonstrated to have a biphasic effect on the estrogenicity in MCF-7 breast cancer cells (25). At the high concentration of kaempferol, diverse cancer growth could be inhibited. Taken together, the current studies indicate that rich consumption of phytoestrogens may be beneficial for prevention of cancer formation based on *in vitro* and *in vivo* results.

This anti-cancer effect of phytoestrogens could be estimated from their chemical structures. Most of their chemical structures appear to be similar to that of an estrogen active form, estradiol. Thus, they may have high binding affinity for ERs, since phytoestrogen and estradiol have competitive binding affinity to ERs. Using a competitive binding assay to measure their binding capacity to ERs, phytroestrogens were examined compared to that of endogenous estradiol. In particular, ERbeta may play more critical roles rather than ERalpha to trigger biological responses caused by phytoestrogens (26). In molecular aspect, flavones and isoflavone could inhibit cytochrome P450 aromatase, which is an essential enzyme in the conversion of androgens to estrogens (27). In our previous study, resveratrol, a natural polyphenolic compound, was shown to inhibit the growth of BG-1 ovarian cancer cells by blocking the interaction of ERalpha signaling pathway and then suppressing cell cycle progression (28).

Anti-cancer effect of kaempferol. Phytoestrogens are classified into isoflavones, coumestans, lignans and coumstans (29). In most of flavonoid structure, the flavon nucleus exists. They have 15 carbon atoms arranged in three rings consist of 2-phenyl-3 hydroxyl-chromonesm. While ring A and B are benzene rings, ring C is a heterocyclic pyran or pyrone (30,31). Kaempferol is a flavonoid, but has different biological properties compared to other flavonoids. Distinct biological properties of kaempferol appear to be derived from its different structure, showing that one of hydroxyl groups in kaempferol is different to other flavonoids shown in Fig. 1 (32).

Kaempferol has been shown to have antioxidant and antineoplastic activities. For instance, kaempferol may reduce proliferation and significantly decrease the expression of vascular endothelial growth factor (VEGF), a marker of angiogenesis, in ovarian cancer cells (33). Kaempferol has been involved to inhibit angiogenesis by suppressing extracellular signal-regulated kinase (ERK)-NFκB-cMyc-p21-VEGF pathway in a cancer cell model (34).

Cells are determined whether they can keep produce or stop through cell cycle, thus the regulation of cell cycle related genes and proteins may be a fundamental procedure for homeostasis (35). Kaempferol has been shown to inhibit cell proliferation in a dose dependent manner by regulating

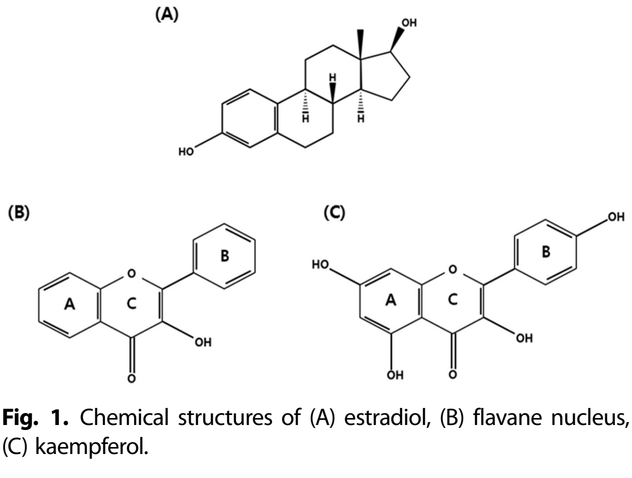

Fig. 1. Chemical structures of (A) estradiol, (B) flavane nucleus, (C) kaempferol.

cyclin-dependent kinase 1 (CDK1) and cyclin B, a marker for transition of G2 to M phase, and by regulating a tumor suppressor gene which plays a key role in cell cycle arrest, p53 or PLK-1, in MCF-7 breast cancer and HeLa cervical cancer cells, respectively (36,37). In addition, kaempferol was demonstrated to suppress cell metastasis through ERK-p38-JNK and AP-1 signaling pathways in U-2 OS human osteosarcoma cells (38). Src kinase has tyrosine kinase activity to regulate mitogen-activated protein kinase (MAPK) activity, which mediate cell cycle and angiogenesis via the inhibition of ultraviolet B (UVB)-induced cyclooxygenase-2 (COX-2). Thus, kaempferol could be a potential therapeutic agent by suppressing these genes against a skin cancer model (38). Based on its molecular mechanism, further *in vivo* study warranties to be examined for a clinical trial to apply beneficial effect of kaempferol.

Physiological role of apoptosis. Human body consists of thirty-three billion of cells, and diverse cell functions maintain their balance. But dual external stimuli, stress and generation of mutant cells may break this balance (39). Program cell death, apoptosis, plays a critical role in maintaining cell functions by removing unnecessary cells, which is a basic procedure to maintain balance by deleting modified or infected cells from our body (40). Cell death may be derived from two major phenomena, necrosis and apoptosis. Necrosis occurs slowly for long period of time by accident, such as physical and chemical stimuli (41). Moisture is absorbed into cells, and then cell membranes are burst and die due to lack of oxygen and bloodstream. Unlike necrosis, apoptosis is a programmed death process against unwanted cells. Cell membrane is distorted shortly and nucleus is disintegrated followed by degradation products which can be disposed of macrophages (42).

In ischemic liver patients, apoptotic bodies appeared to be different from necrotic bodies in morphology. Thus, new form of death to shrinkage necrosis was found at first time, and it was named as apoptosis at the following year (43). Apoptosis was derived from Greek letter of falling off, implying overall natural metabolic process like fallen leaves. While necrosis always occur in pathological cells, apoptosis can be also observed in pathological and normal cells (44). In embryonic development, many unnecessary cells are removed through apoptosis, for example, when fingers are formed in embryonic development, intervals cells are removed to get entire function. A similar process happened when tadpole's tail is missing because its function is no longer required after growing (45). In this situation, abnormal or unnecessary cells could be dead by apoptosis. When cells are exposed to stress by chemical substance and internal stimuli, normal cells turn into mutant and finally changed as cancer cells (45). In this case, our immune system could find out pathological or abnormal cells to remove, indicating that homeostasis is maintained and regulated by apoptosis (45).

To measure apoptosis, traditional cell-based methods have been developed, i.e., a DNA laddering assay. When programmed cell death begins, DNAs in nuclei are regularly fragmented to 80~200 bp, which can be visualized on agarose gel (46). Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling of DNA fragments (TUNEL) and in situ end labeling (ISEL) staining methods have been developed to detect apoptosis in cells and tissues (47). These methods can be also used together with standard flow cytometric staining methods related with cell death using various cellular parameters, including cell cycle and phenotypes (48). TUNEL assay not only has a merit to maintain tissue structure but also observe apoptotic body in individual cells. In addition, we can use microscopy to observe cytological appearance changes, i.e., membrane blebbing and nuclear condensation. A MTT/XTT enzyme assay and caspase activity assay can be employed to measure apoptosis in cells or tissues (49).

Effect of kaempferol on apoptosis. In extrinsic pathway, there are death receptors in cell surface, and they may recognize substances which induce death and penetrate to inner cytoplasm. Death receptors include tumor necrosis factor (TNF), FAS and TRAIL (50). Kaempferol down-regulates TNF-α production in aged rat gingival tissues via the inhibition of transcription factor nuclear factor kappa B (NF-κB) (51). Unlikely quercetin, kaempferol appears to be effective in both osteoblasts and osteoclasts by antagonizing TNF-α and receptor activator of NF-κB ligand receptor activator of NF-κB ligand (RANKL) (52). TRAIL has been shown to induce apoptosis in SW480 human colon cancer cells (53). It was demonstrated that kaempferol significantly up-regulate TRAIL receptors, indicating that kaempferol may be an effective factor in the treatment for TRAIL related-immune deficient disease (54). In human glioma cells, kaempferol also sensitizes to TRAIL-mediated apoptosis by proteasomal degradation of survivin (55).

Caspases are a zymogen which is an element in the induction of apoptosis as a downstream signaling pathway. A difference of these caspase pathways is irreversible compared to other post-translational modifications (56). When procaspases convert into caspases, they have enzymatic activity in the induction of apoptosis (57). In apoptosis, kaempferol slightly induced caspase 3 activity in leukemia HL-60 cells and oral cavity cancer cells (36,58). An intrinsic pathway mainly occurred in mitochondria by intracellular factors, i.e., apoptotic genes; p53, PUMA, NOXA, Bax, BCL-2, Apaf-1, caspase 9 and cytochrome c etc. (59,60). In A549 lung cancer cells, kaempferol up-regulated pro-apoptotic bax and bad genes, while it down-regulated anti-apoptotic bcl-2 and bcl-xL expression (61).

Nitric oxide (NO) or reactive products modulate apoptotic signals. They regulate caspase 3-like proteases ultimately to control activity of protease (62). In addition, NO and reactive products have also influenced TNF- or FAS signals. NO has been shown to possess different functions depending on the type of cells and state (63). They kill cancer cells and protect normal cells by plotting between normal and cancer cells (64). NO synthesis is induced by isoforms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2). In addition, NO production is regulated by reactive C-protein (CRP) and NF-kB activation (65). In some diseases, iNOS and COX-2 can be overexpressed. In human hepatocyte-derived cell line, Chang liver cells, kaempferol has been shown to suppress the protein levels of iNOS, COX2, CRP and NF-κB at all concentrations of 5 to 200 μM (66). To measure NO synthesis by a drug in human and animals, it is more complicated in depending on various factors, thus real-time PCR can be used to directly measure NO synthesis products (67). In the previous study, kaempferol was shown to inhibit NO production in a rat model (68).

CONCLUSION

As an alternative method for HRT, the effects of phytoestrogens have been explored in the previous studies. However their effects appear to be diverse depending on their concentrations. A high concentration of 12 phytoestrogen mixture has been shown to have an estrogenic activity in Sprague-Dawley rats (69). As a phytoestrogen, kaempferol has also been determined to have a biphasic effect. For instance, kaempferol has an anti-cancer activity at higher concentrations, while it has a pro-cancer activity at lower concentrations (25). Thus, the concentration of its dosage should be considered for the application to human cancer treatments. In addition, the signaling pathways caused by kaempferol should be clarified in a cell model, for instance, kaempferol may be involved in the induction of apoptosis in many cancer cell types, implying that it may have an anticancer activity through intrinsic and extrinsic apoptotic pathways.

More detailed studies should be performed to confirm anti-cancer effect(s) of kaempferol for clinical use. An interaction caused by several factors, such as sex, concentration, and immune state, needs to be considered as well. Although many studies have been done to clarify the exact role(s) of kaempferol in *in vitro* and animal models until now, more detailed studies should be carried out to clarifying its underlying mechanism(s) for the clinical use.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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