Function of flavonoids on different types of programmed cell death and its mechanism: a review

Preethi Vetrivel1,△, Seong Min Kim1,△, Venu Venkatarama Gowda Saralamma1, Sang Eun Ha1, Eun Hee Kim2, Tae Sun Min3✉, Gon Sup Kim1✉

1Research Institute of Life Science and College of Veterinary Medicine, Gyeongsang National University, Jinju, Gyeongsangnam-do 52828, Republic of Korea; 2Department Institute of Women’s Health Care, Jinju, Gyeongsangnam-do 52818, Republic of Korea; 3Faculty of Biotechnology, Sustainable Agriculture Research Institute, Jeju National University, Jeju 63243, Republic of Korea.

Abstract

Cell death in the living system plays a vital role in maintaining the homeostasis and balancing the cell count in the body. Programmed cell death (PCD) is a crucial component of several development and defense mechanisms. PCD is also important in terms of aging which avoids the accumulation of cellular damage by maintaining cell division. Depending on the execution of cell death and its role in destruction, PCD is categorized into several subtypes. The major different forms of PCD in animals are apoptosis, autophagy and necrosis, which can be distinct in morphological terms. More intense investigations of cell death have given close insight showing other important types of cellular destruction and their pivotal roles in treating disease conditions like cancer. Flavonoids have been acquired a great interest for disease therapies and chemoprevention through activation of several PCD mechanisms. The significant potential of natural flavonoids in the induction of distinct signaling cascades is being a massive approach for targeting uncontrolled cell growth. For these reasons, understanding PCD mechanisms is a promising approach for the interventions in treating cancer. Thus, it is intriguing that understanding the different forms of PCD mechanism induced by flavonoids with more accurate descriptions on the biochemical and cellular processes are gaining more significance in cancer research. Here, we provide a brief overview on the different types of PCD and aim to discuss the functional role of flavonoids in promoting different types of cell death as well as an extensive brief review on their mechanism of action has been highlighted.

Keywords: programmed cell death, apoptosis, necrosis, autophagy, anoikis

Introduction

Cells in multicellular organisms are a highly organized community that is tightly regulated by the rate of cell division and controlled by cell death mechanism. The cells that are no longer required for...
the body function or in defected condition tends to commit suicide by activating intracellular death program called programmed cell death (PCD). PCD is being defined as the sequence of events that leads to the organized and controlled destruction of cells[1]. It is carried out as a continual incidence of biological processes which confers an advantage in a life-cycle of an organism. The mechanism of cell death serves two main functions: (i) as an antagonist in mitotic cell division maintaining controlled elimination of excessive cells; and (ii) as a defense mechanism in diseases like cancer by discarding the mutated cells[2]. The concept of cell death is significant to elucidate the response of various tissues to toxic injury as well as in the mechanism of carcinogenesis. The induction of cell death via several types of PCD in addition to the inhibition of cell proliferation is now becoming a major strategic approach for cancer prevention and treatment[1].

Flavonoids are an important class of natural compounds from plant sources that belongs to the class of secondary metabolites with miscellaneous biochemical and functional effects. They are the major constituents of polyphenolic compounds that are abundantly found in sources of several vegetables, fruits like apples, berries, grapes, and their products like green tea, red wine, cocoa and many more. There are different groups of flavonoids namely, anthocyanins, flavanols, flavones, flavonols, flavonones and isoflavones. Each of these subgroups and each type of flavonoid carries its own mode of action and benefits towards induction of PCD[1–4].

Functionally flavonoids possess a broad spectrum of health promoting effects with significant anti-inflammatory, anti-tumor, anti-mutagenic and anti-oxidant activities coupled with their capacity to modulate key cellular enzyme functions. The biological activities of flavonoids lead to inactivation of oxygen radicals, binding of electrophiles and induction of some protective enzymes that are vital in promoting their anti-oxidative property. Flavanols possess the ability to induce conjugating enzymes like uridine diphosphate glucuronosyltransferase (UDP-GT) that inactivates the free radicals and reactive oxygen species (ROS) which thereby prevents the formation of cancers. The molecular mechanism of anti-proliferative effect of flavonoids has shown to involve the inhibition of growth promoting oxidants and ROS, which are the major catalysts of tumor progression[5]. These properties make them favorable to play potential protective role in various chronic diseases including cancers. Also, the fortunate nature of flavonoid in the treatment of cancer is their ability to induce cell death in cancer cells with specific cytotoxicity and with minimal cytotoxic effect towards normal cells. Flavonoids play a crucial role in eliminating cancer cells by inducing cancer specific PCD with lower cytotoxicity, which gives a promising and most realistic chemo-preventive approach for malignant disorders[6–7].

Over the past few years, the extensive action of flavonoids on chronic disease has been demonstrated their multiple roles in signal transduction pathways related to cellular proliferation, differentiation, cell-cycle progression, PCD, inflammation and metastasis[8–9]. However, the molecular mechanisms of action of flavonoids are not completely characterized and elucidated. In the present review, attempts have been made to discuss the induction of different types of PCD by flavonoids and their mechanisms in detail.

Different modes of PCD

Cell death in multicellular organisms occurs in different mechanisms including apoptosis, autophagy, necrosis, and total of 11 forms of PCD according to the Nomenclature Committee on Cell Death[5]. The mode of cell death can be generally categorized based on its morphological characteristics, enzymological role and functional characteristics into different types[10–11]. The most common forms of PCD are divided on the criteria as: apoptosis that requires energy, and necrotic cell death that do not require energy. Apoptosis usually occurs in response to extrinsic or intrinsic death signals, such as death signals attracted by receptors, cytochrome C released from the mitochondria. In contrast, the necrotic cell death mechanism is of passive type characterized by cells swelling. Depending on the type of cells, and the death signals, the apoptotic and necrotic mechanisms can overlap[12]. Autophagy is another form of catabolic pathway of cell death that allows cells to degrade their own components by the formation of autophagosomes, degrading cytoplasmic contents and chromatin condensation. Similarly, necrosis is a type of PCD that characterized by a disruption of plasma membrane leading to the release of cellular components and inducing inflammatory responses[13].

Apoptosis

Apoptosis is a critical cell death mechanism featured in an orderly fashion of events with specific biochemical pathways leading to the termination of a cell's life[14]. Morphologically, apoptotic cell death initiates at the cell nucleus level, causing chromatin condensation and subsequently to its shrinkage and fragmentation of DNA. Biochemically apoptotic cell
death is mediated by proteolytic enzymes called caspases, which trigger cell death by cleaving specific proteins in the cytoplasm and nucleus[3]. Apoptotic signaling pathways mainly function through two major pathways – the intrinsic mitochondrial mediated pathway and the extrinsic death receptor pathway. The extrinsic apoptotic pathway is defined by caspase-dependent, whereas the intrinsic apoptotic pathway is acquired through either caspase-dependent or caspase-independent signaling[15].

The extrinsic mediated pathway is generally mediated via the specific transmembrane receptors that belongs to the tumor necrosis factor (TNF) receptor family. The mode of initiation involves the binding of ligands such as Fas ligand, TNF-α or TNF-related apoptosis inducing ligand (TRAIL) to the death receptors namely Fas (APO-1 or CD95), TNF receptor-1 and TRAIL receptor 1 or 2, leading to the oligomerization of cell surface receptor causing activation of signaling cascades culminating in apoptotic cell death. The intrinsic mediated pathway is activated in response to cellular stress like DNA damage, oxidative stress, calcium overload, endoplasmic reticulum (ER) stress. The mechanism of action is characterized by multiple events: initially by the decedence of the mitochondrial membrane potential ($\Delta \Psi_m$) leading to the release of proapoptotic signals like cytochrome C, apoptosis-inducing factor (AIF), and other activators of caspases into the cytosol. The cytosolic cytochrome C further triggers the formation of the multiprotein complex called apoptosome possessing cytochrome C, Apaf-1 (apoptosis platform for caspase activation) and procaspase-9. This complex in turn leads to the activation of downstream caspases thereby resulting in apoptosis (Fig. 1)[16–17].

**Autophagy**

Autophagy is the second most common mode of PCD and also known as type 2 PCD, which is a self-destructive mechanism that balances the sources of energy at crucial times during development and in response to nutrient stress. The mode of autophagic cell death can be selective in removing certain organelles like ribosomes, aggregated proteins as well as in eliminating the intracellular pathogens[18].

The mechanism of autophagy is characterized by the separation of cytoplasmic material within autophagosomes that results in bulk degradation by lysosomes. The auto phagosomes with the degrading acidic enzymes lysosomal hydrolases catalyzes the contents in the vesicles which marks the completion of the autophagic pathway (Fig. 2)[19–20]. Apart from the lysosomal degradation, autophagic machinery also involves the responses to various external stimuli such as microorganisms like pathogenicity during bacterial infection. In addition to elimination of intracellular aggregates and pathogenicity, autophagy also promotes effective cellular senescence giving a key role in preventing diseases such as cancer[21]. Controversially, autophagy greatly improves the fitness of cancer cells under stressful conditions and, thus, attenuates apoptosis and necroptosis. Whereas, excess autophagy induces the death of metastasizing cells, thus, tight regulation of autophagy will greatly contribute to improve the treatment strategies in cancer[22–23].

**Fig. 1 Schematic representation of the key events in the mechanism of apoptosis.** In the extrinsic apoptotic pathway, the binding of Fasl, TNF-α or TRAIL receptors activates the procaspase-8 to caspase-8 followed by apoptosis. In the intrinsic apoptotic pathway, oxidative stress or toxic damage destroys the mitochondrial membrane permeability causing the release of cytochrome C which activates caspase-9 to caspase-3 followed by apoptosis. DDR4: death receptor 4; DDR5: death receptor 5; FADD: Fas-associated protein with death domain; TRADD: TNF receptor-associated death domain.
Necrosis/Necroptosis

The primary form of cell death featured by uncontrolled cell dying process that occurs in response to infection, toxins or injury to the cells result in necrosis\[13\]. In this type of cell death, the cells upon external stimuli undergo an increase in cell volume owing to swelling and rupture of membranes ending up in loss of intracellular contents. Necrosis initially considered as an accidental uncontrolled form of cell death, but increasing evidences suggests the execution of necrotic cell death are regulated by various signal transduction pathways and catabolic mechanisms. Regulated necrosis is termed "programmed necrosis" or "necroptosis" to distinguish it from necrosis caused by physical damage\[13,21\].

The mode of action of necrosis is induced by death receptors like TNFR1 stimulation which triggers on the basis of kinase activity of receptor interacting protein (RIP). The two distinct forms of RIP kinase proteins RIPK1 and RIPK3 are present with Fas-associated protein with death domain (FADD), caspase-8 and TNF receptor-associated death domain-containing protein (TRADD) in the necrosome which induces necroptosis. Normally activated caspase-8 triggers apoptosis, but in case of absence of caspase the phosphorylation of RIPK1 and RIPK3 leads to necrotic cell death (Fig. 3)\[17,24\].

Anoikis

Anoikis mediated PCD is induced upon the detachment of cell from the extracellular matrix that behaves critically in adherent-independent cell growth. The initiation and execution of anoikis are mediated through different pathways which converge at the end in the activation of caspases. The induction activates through the interplay of the two apoptotic pathways, the intrinsic and extrinsic pathway\[25\] (Fig. 4). The perturbation of mitochondria and triggering of cell death receptors leads to the activation of cascades of caspase proteins which in turn provokes downstream regulators culminating the activation of endonucleases followed by DNA fragmentation and cell death\[26–27\].

The molecular events of anoikis mediated cell death are initiated in the absence of attachment of extracellular matrix due to cell adhesion to inappropriate locations. This critical mechanism of PCD features the loss of binding of substrate of the cells. It is also an important defense mechanism that helps to prevent detached cells to adhere to the new matrix at incorrect sites of growth. Thus anoikis is an important mode of cell death mechanism which serves as an emerging hallmarks of cancer cells leading to the formation of metastasis\[28–29\].

Flavonoids in induction of PCD

Flavonoids have been shown to trigger different PCD through modulation of several key events in terms of cellular signaling mechanism. It has been reported that flavonoids are involved in exerting regulatory events in cells with important signal cascades such as cyclin-dependent kinases (CDKs), caspases, Bcl-2 family members, epidermal growth factor, epidermal growth factor receptor, PI3K/Akt, MAPK, and NF-κB\[6,30\].
Activation of apoptotic cell death by flavonoids

The potential features of flavonoids to inhibit cell proliferation and their ability to induce apoptosis in human cancer cells has stimulated intense interest as active anti-cancer agents. Increasing evidences reveals that the activation of apoptosis associated with cell cycle arrest by flavonoids as many cancer therapeutic agents. Our previous studies have indicated and confirmed that flavonoids isolated from Korean Citrus aurantium L. effectively inhibited the proliferation of cancer cells through arresting cell cycle and inducing apoptosis in gastric cancer cells via the modulation of cell-cycle-related proteins, Cdc 2, cyclin B1, and Cdc25C and provoking of apoptosis via up-regulation of caspase-3 activity and followed by the cleavage of PARP[31]. Flavonoids isolated from Citrus platymamma induced mitochondrial-dependent apoptosis in AGS cells by the modulation of PI3K/Akt and MAPK pathways and regulated the protein expression levels of cyclin B1, CDK1 and Cdc25C. Whereas same C. platymamma flavonoid extract induced caspase-dependent cell death by up-regulation of the Bax/Bcl-xL ratio and the protein expression levels of cyclin B1, CDK1 and Cdc25C were modulated in A549 human lung cancer cells showing that the mechanism of action of flavonoids towards cancer are unique to each cancer cells[32]. The flavonoid extract from Citrus species has been proven to possess several flavonoids and the major ones are hesperidin, nobiletin, sinensetin, naringin, and poncirin which have been individually proven to exhibit anti-inflammatory, antioxidant and anticancer properties by regulating various

**Fig. 3** Mode of action of cell death mediated by necroptosis. The mechanism of necroptosis involves the activation of FAS/TRAIL-R, TNFR1 and TLR3/4 signaling complex that subsequently results in the phosphorylation of RIPK1 and RIPK3 leading to necrotic cell death. MLKL: mixed lineage kinase domain-like protein.

**Fig. 4** Molecular events that occur in anoikis mediated PCD. The activation of different anoikis pathways with respect to lack of ECM contact. The extrinsic pathway is activated by increased expression of Fas receptors that triggers caspase-8 and the intrinsic pathway is induced by the up-regulation of pro-apoptotic molecules such as Bad, Bid, Puma, Hrk, Bmf and Noxa triggering caspase-9. Both the pathways converge at caspase-3 activation promoting anoikis. ECM: extracellular matrix; FADD: Fas-associated protein with death domain.
pathways\textsuperscript{33–36}. The most familiar flavonoids quercetin and tangeretin has shown induction of apoptosis via the mechanism of activation of p53 in cervical and gastric cancer cells\textsuperscript{37–38}. Several in vitro studies on the flavonoid myricetin has been proven to show cytotoxic effect on colon, esophageal, and breast cancer cells, as well as leukemia and melanoma cells. The evidences suggest that the mitochondrial apoptosis pathway has been activated with increased expression of caspase-9 and caspase-3 levels\textsuperscript{39–40}.

**Activation of autophagic cell death by flavonoids**

The modulation of autophagy plays dual roles by suppressing tumor growth and promoting cell growth under stressful conditions in many cancers. Studies suggest that autophagy is a regulator of many tumor suppressor and oncogenes genes. To the contrary, other studies have shown that autophagy is involved in both the development and the promotion of tumorigenesis and inhibition of cancer. Some of the flavonoids shown anticancer effect by inducing excess autophagy in several cancer cells by regulating oncogenes leading to cancer cell death\textsuperscript{41}. Naringin found in citrus fruits have been also reported to exert a variety of anti-tumor and anti-inflammatory effects by the induction of apoptosis in cancer cells. Studies reported that the inhibitory mechanism of naringin in AGS human gastric cancer cells via induction of autophagy by down regulation of PI3K/Akt/mTOR signaling cascades\textsuperscript{33}. A study on the effect of curcumin and quercetin has shown to induce autophagic cell death in myeloid leukemia cells and epithelial cancer cells via the down-regulation of Bcl-2 protein. Similarly, marked decrease in the levels of protein p62, which is a cargo receptor for autophagic degradation and indication of autophagic induction, has been observed extensively in previous studies. The PI3K/Akt/mTOR pathway has been known for its activation frequently in human cancers, which is an attractive target for anticancer drug development. Increasing evidences indicate the inhibition of this signaling pathway by several flavonoids (procyanidins, hesperidin) leading to autophagic cell death by the suppression of PI3K-p110 expression in several cancer cells\textsuperscript{31}. Autophagy can inhibit apoptotic cell death by promoting cell survival, in contrast, autophagy and apoptosis can cooperate as partners to induce cell death. Our study on anticancer effect of pectolinaringenin, a flavonoid compound induces G2/M arrest, apoptosis, and autophagy by regulating PI3K/Akt/mTOR pathway in human gastric cancer cells\textsuperscript{42},

**Activation of necrosis, anoikis and other forms of PCD by flavonoids**

Most in vitro studies conducted till date have focused on the effect of individual precursor flavonoids on inflammatory mediators in case of necrosis. Described models in melanoma cells have shown to trigger necrotic cell death in distinct forms, either via activation of Bim or by FADD associated mechanism upon treatment with flavonoids\textsuperscript{43}. Anthraquinone-based derivatives has exhibited great potential as necrosis-avid agents initiating tumor necrosis with low cytotoxicity to normal cells\textsuperscript{44}. The active mechanisms of flavonoids like apigenin, kaempferol, chrysirin has proven their effects on the NF-κB pathway via inhibition of cytokine TNF-α playing host defense against infections owing to necrotic cell death in cancer cells\textsuperscript{44}.

The evaluated potential of bioactive flavonoids in mediating cancer cell death via anoikis in treating metastatic cancer progression has been studied extensively. The prognosis of integrin-mediated cell detachment has been shown to require for cancer cell migration and metastasis. Thus, targeting integrin-mediated ECM pathways via focal adhesion kinase (FAK) proteins is relatively effective in controlling anoikis mediated PCD\textsuperscript{45}.

The epithelial cancer cells adhere to the ECM and forms binding with focal adhesion proteins mediated by integrins and thereby promotes protein tyrosine kinase 2 (PTK2) to induce proliferation through downstream signal transduction. The modulation of PTK2 activity owing to its downregulation can promote cancer cell anoikis\textsuperscript{46}. Recent reports demonstrated that dietary flavonoid apigenin has modulated the signaling pathways, including PI3K/Akt, MAPK/ERK, JAK/STAT, and NF-κB in suppressing cell migration in human lung cancer cell line through anoikis\textsuperscript{47}. In other studies, the ERK signaling pathway has been inhibited by apigenin along with protein kinases, say FAKs inducing anoikis on prostate cancer cells\textsuperscript{48}. Curcumin from turmeric has shown to sensitize anoikis in lung cancer cells by mediating Bcl-2 down regulation. In addition, the compounds from a mixture of flavonoids extracted from Korean Citrus aurantium has reported to induce anoikis involving caspase-3 proteins. Mounting evidences have demonstrated the role of flavonoids in inhibiting the anchorage-independent growth of cancer cells by down-regulation of Mcl-1 protein\textsuperscript{49}.

Interestingly, these earlier reports have firmly showed the role of flavonoids as a potential anti-
Flavonoids in programmed cell death

metastatic agent by sensitizing anoikis-resistance by suppressing survival proteins p-ERK along with the anti-apoptotic proteins Bcl-2 and Mcl-1 respectively.[30] An alternative non-apoptotic cell death pathway may contribute to the development of new therapies.

Paraptosis is a type of non-apoptotic PCD that has been discovered in recent years. It is characterized by extensive cytoplasmic vacuolation due to the dilation of the ER and/or mitochondria. Studies on monomer compound hesperidin suggests that hesperidin-induced mitochondrial Ca\(^{2+}\) overload leads to increase ROS production and loss of \(\Delta \Psi_m\), which finally leads to paraptotic cell death in HepG2 human hepatoblastoma cells[14]. Morusin, a prenylated flavonoid isolated from the root bark of Morus australis, induced mitochondrial Ca\(^{2+}\) influx through voltage-dependent anion channel and subsequent mitochondrial Ca\(^{2+}\) overload which resulted in depletion of \(\Delta \Psi_m\) and increased generation of ROS, leading to paraptosis-like cell death in epithelial ovarian cancer[37].

Conclusion

The use of natural constituents as promising chemopreventive agent against cancer has been extensively studied in the last two decades. Studies have suggested that the use of anti-cancer phytochemicals with different mechanisms or modes of action may be more effective in treating the diseases and limiting the side-effects. Implication of flavonoids in the treatment for cancer and also as dietary sources would be significant towards the prevention and treatment of disease like cancer. With the reported studies and evidences discussed in this review, it gives a better perspective of the mode of action of flavonoids on the activation of different forms of cell death by regulating key pathways. The efficacy of flavonoids to act on target genes in the chemoprevention studies has made them attractive candidates for new conventional therapies.

The proposed anticancer mechanisms of flavonoids studied in recent years are mainly involved in the inhibition of cell proliferation, inflammation, invasion, metastasis and activation of PCD. However, further more studies need to be carried out to develop biomarkers for flavonoid treatment in cancer and intake of flavonoid-rich diets may influence gene regulation for cancer prevention.

Acknowledgments

The research was supported by the Program of National Research Foundation of Korea through the Ministry of Education.

References


