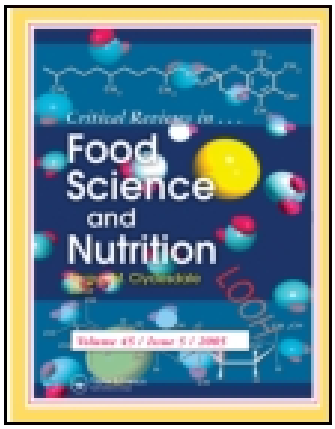

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REVIEW

Differential Modulation of Apoptotic Processes by Proanthocyanidins as a Dietary Strategy for Delaying Chronic Pathologies

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Apoptosis is a biological process necessary for maintaining cellular homeostasis. Several diseases can result if it is deregulated. For example, inhibition of apoptotic signaling pathways is linked to the survival of pathological cells, which contributes to cancer, whereas excessive apoptosis is linked to neurodegenerative diseases, partially via oxidative stress. The activation or restoration of apoptosis via extrinsic or intrinsic pathways combined with cell signaling pathways triggered by reactive oxygen species (ROS) formation is considered a key strategy by which bioactive foods can exert their health effects. Proanthocyanidins, a class of flavonoids naturally found in fruits, vegetables, and beverages, have attracted a great deal of attention not only because they are strong antioxidants but also because they appear to exert a different modulation of apoptosis, stimulating apoptosis in damaged cells, thus preventing cancer or reducing apoptosis in healthy cells, and as a result, preserving the integrity of normal cells and protecting against neurodegenerative diseases. Therefore, proanthocyanidins could provide a defense against apoptosis induced by oxidative stress or directly inhibit apoptosis, and they could also provide a promising treatment for a variety of diseases. Emerging data suggest that proanthocyanidins, especially those that humans can be persuaded to consume, may be used to prevent and manage cancer and mental disorders.

Keywords Apoptosis, proanthocyanidins, cell signaling, chronic disease, modulation

1. INTRODUCTION

The process of programmed cell death, or apoptosis, is a normal physiological mechanism that occurs during the development of multicellular organisms and continues throughout adult life. Embryogenesis combines apoptosis with cell proliferation to shape tissues and organs, whereas in adults, apoptotic processes are considered vital for maintaining cell turnover (i.e., balance between proliferation and cell death) and proper development (Khan et al., 2007).

Apoptotic cellular response to a variety of stimuli is a controlled process that differs from necrosis in that it minimizes the leakage of cellular constituents from dying cells and does not affect neighboring cells or induce an inflammatory reaction.

Apoptosis is a finely balanced process, and any disruption in tissue or organ homeostasis by deregulated apoptotic mechanisms can lead to numerous pathological conditions. In fact, the pathogenesis of stroke, neurodegenerative diseases, and autoimmune processes is closely connected with excessive apoptotic processes, whereas inhibition of apoptosis can contribute to many types of cancers by enhancing uncontrolled cell growth or inhibiting antigrowth signals, finally resulting in organ dysfunction (Finkel, 2001). Whether a cell survives or dies by apoptosis is determined by the balance between proapoptotic (stress or death) signals and antiapoptotic (mitogenic or survival) signals within and around the cell (O'Brien and Kirby, 2008).

In recent years, an increasing number of studies have investigated how different components of the diet interact at the molecular level to determine their pro- or antiapoptotic effects. Plant

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polyphenolics have been shown to induce growth arrest and apoptosis by regulating multiple signaling pathways (Shankar et al., 2007) in which reactive oxygen species (ROS) seem to be indispensable (Fang et al., 2007). Dietary factors exert their action at different pathological stages by their antioxidant and/or free-radical-scavenging abilities, which might be partially related to the induction of apoptosis (Aparicio-Fernández et al., 2008).

Indeed, proanthocyanidins, a family of polymeric flavanols (flavan-3-ols), have demonstrated not only anticancer properties by inducing apoptosis or inhibiting cell proliferation but also neuroprotective effects by inversely regulating apoptotic mechanisms (de la Iglesia et al., 2010). These effects are thought to complement one another to prevent cell injuries induced by oxidative stress by potentiating endogenous oxidative defense mechanisms through the well-described antioxidant effect of dietary proanthocyanidins.

Understanding the proapoptotic and antiapoptotic cell pathways and how they are affected by proanthocyanidins is important for understanding the mechanisms of many life-altering diseases in humans and animals and realizing the potential for novel therapeutics based on polyphenolic-rich food matrix consumption.

The purpose of this review is to outline the concept of dual modulation of apoptosis by proanthocyanidins as an important target for dietary strategy to prevent, delay, or improve the development of cancer and neurodegenerative diseases or the alteration of homeostasis.

2. MORPHOLOGICAL AND BIOCHEMICAL FEATURES OF APOPTOSIS

Typically, apoptosis is an active gene-directed process of cell death that occurs under a variety of conditions. Morphological changes characteristic of apoptotic events include cell shrinkage, membrane blebbing, chromatin condensation, and formation of a DNA ladder with multiple fragments caused by internucleosomal DNA cleavage. These morphological hallmarks are accompanied by biochemical features such as increased mitochondrial membrane permeability, release of proapoptotic proteins, formation of apoptotic bodies, and the appearance of phosphatidyl serine (PS) on the cell membrane surface (Perl et al., 2005).

3. APOPTOTIC PATHWAYS

There are two major pathways that initiate apoptosis: the extrinsic [death receptor (DR)-mediated] and the intrinsic (mitochondria-mediated) pathway, as illustrated in Figure 1. Both pathways activate caspase cascades, which play a central role in the execution phase of apoptosis (O'Brien and Kirby, 2008).

Briefly, the extrinsic pathway of apoptosis is activated at the cell surface when a specific ligand binds to its corresponding cell-surface DR. The interaction between FasL or tumor necrosis factor (TNF)-α with Fas or TNF receptor-associated factor (TRAF) family proteins results in the formation of a supramolecular complex called the death-inducing signaling complex (DISC). This complex recruits caspase-8 and 10 via Fas-associated protein with death domain (FADD) or TNF receptor type 1-associated death domain protein (TRADD), respectively, and directly cleaves and activates caspase-3, an apoptotic executor caspase.

As shown in Figure 1, the intrinsic pathway involves the collapse of the mitochondrial membrane, accompanied by the translocation of cytochrome C to the cytoplasm along with other proapoptotic factors such as endonuclease G and apoptosis-inducing factor (AIF).

The release of cytochrome C, apoptotic protease-activating factor 1 (Apaf-1), and procaspase-9 forms a supramolecular complex known as the apoptosome that activates caspase-9, which activates caspase-3, which in turn cleaves poly (ADP-ribose) polymerase (PARP) and triggers DNA fragmentation.

The caspase pathway is regulated by inhibitor of apoptosis protein (IAP), which is neutralized in apoptotic events by the second mitochondria-derived activator of caspase (Smac) and the direct IAP-binding protein with low pI (DIABLO) released from mitochondria. How successful the pathway is at inducing apoptosis depends on the balance between the proapoptotic and the antiapoptotic members of the Bcl-2 superfamily of proteins (Li et al., 2004b).

Furthermore, there is a third and little understood apoptotic pathway known as the endoplasmic reticulum (ER) pathway, which is involved in apoptosis induced by UV radiation and free radicals. The relationship between apoptosis and oxidative stress, if any, makes the study of phytochemicals with antioxidant potential even more interesting (Morishima et al., 2002; Zong et al., 2003; Rao et al., 2004).

4. REGULATION OF APOPTOSIS BY CELLULAR SIGNALING PATHWAYS

Apoptosis is regulated by several genes in both the extrinsic and the intrinsic pathway, some of which (Bax, Bcl-xs, c-myc, p53, cfos) promote apoptosis, whereas others inhibit it (Bcl-2, Bcl-xL). In addition to these main pathways, mitogenic and stress-related pathways are also involved in regulating apoptosis. In fact, the three major mitogen-activated protein kinases (MAPKs)—extracellular-signal-regulated protein kinase (ERK), c-Jun N-terminal protein kinase (JNK), and p38 MAPK—are involved in early signaling mechanisms. Disruption of the MAPK signaling pathway leads to pathophysiological changes, including oncogenesis and apoptosis (Wada and Penninger, 2004), and because JNK/ERK/p38 phosphorylation regulates cell proliferation, differentiation, and apoptosis, the net balance between cytoprotective (e.g., ERK) and stress-related (e.g., JNK) signaling may play a critical role in cell survival and death decisions (Tournier et al., 2000; Johnson and Lapadat, 2002; Watanabe et al., 2002; Li et al., 2008). The

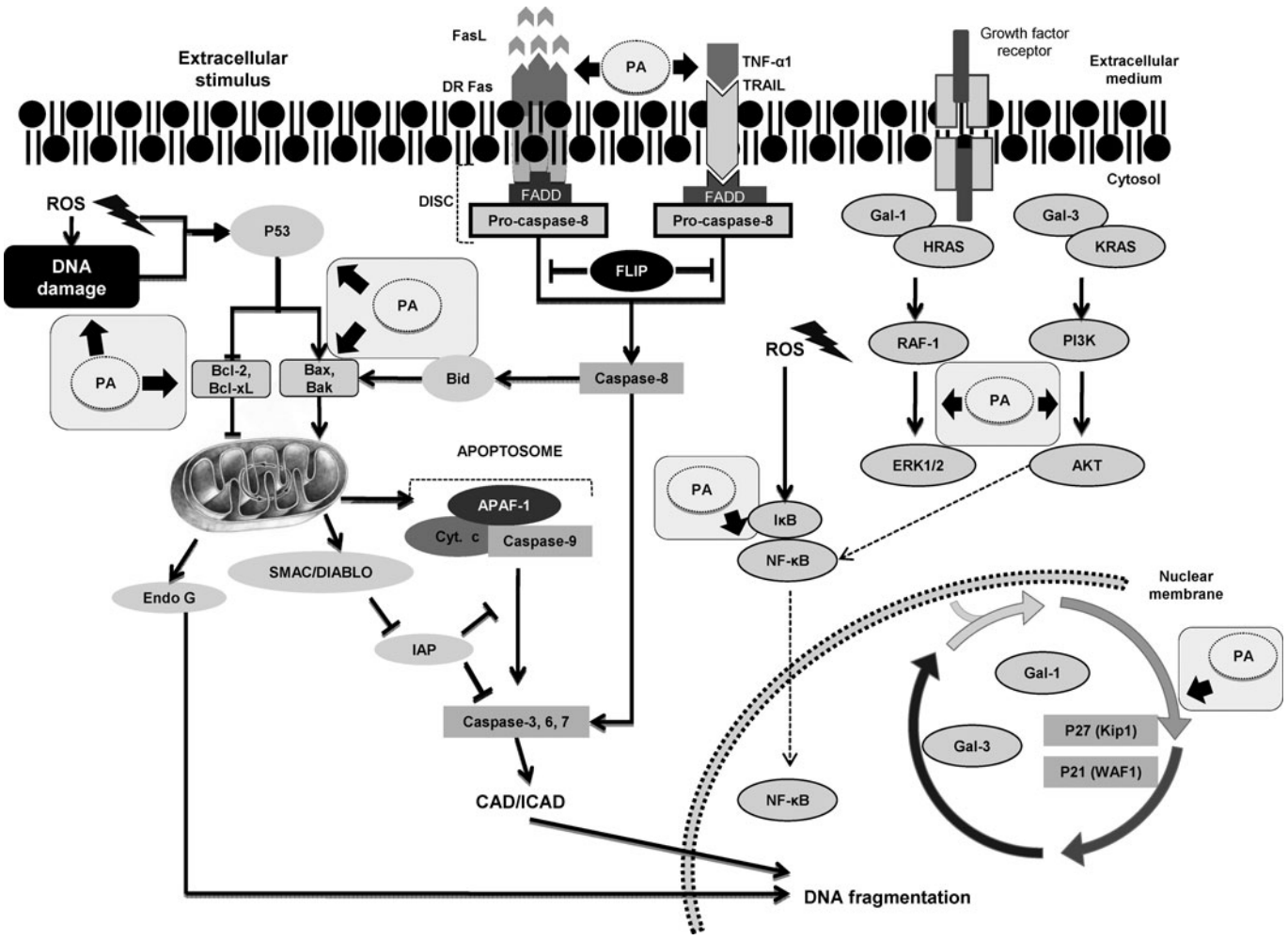

Figure 1 Targets of bimodulated apoptosis by proanthocyanidins (PA) on intrinsic, extrinsic, and mitogenic-related pathways. Bcl-2 = B-cell lymphoma-2, IAP = inhibitor of apoptosis protein, Apaf-1 = apoptosis-activating factor-1, Smac = second mitochondrial-derived activator of caspases, DIABLO = director inhibitor of apoptosis-binding protein with low pI, BH3 = Bcl homology- 3, tBid = truncated Bid, EndoG = endonuclease G, AIF = apoptosis-inducing factor, Bax = Bcl-2-associated protein x, Bak = Bcl-2-associated protein k, FasL = Fas ligand, TNFR = tumor necrosis factor receptor, FADD = Fas-associated death domain, TRADD = TNF-associated death domain, c-FLIP = FLICE-like inhibitory protein, DISC = death-inducing signaling complex.

serine/threonine protein kinase, protein kinase B (a member of the PI3-K pathway), is a crucial regulator of widely divergent cellular processes, including apoptosis (programmed cell death), cell proliferation, differentiation, and metabolism. Its disruption has been frequently observed in several human cancers, and it appears to play an important role in cancer progression. The PI3-K signaling pathway should therefore be considered a potential target for chemotherapy (Luo et al., 2003; Stephens et al., 2005).

5. APOPTOSIS AND DISEASES

Disorders of apoptotic regulatory mechanisms are involved in the development of various diseases, such as AIDS, neurodegenerative disorders, and many types of cancer (Roy et al., 2005; Chen et al., 2010).

In neurodegenerative diseases, normal cells undergo excessive apoptosis, which leads to the progressive loss of neurons. In contrast, cancer is characterized by too little apoptosis; cells ignore normal signals that regulate growth and proliferation. In the case of mutated cancer cells, the lack of control points in cell cycle progression leads to excessive proliferation and finally to the formation of tumors and cancer.

Therefore, activation or restoration of apoptosis by dietary factors could be a key strategy to prevent, delay, or treat chronic pathologies. Several observations indicate that apoptosis may be mediated in a cell-specific manner, in which the cells of one biological compartment are induced to die, while others are not (Roy et al., 2005; Martin, 2006; Chen et al., 2010).

6. PROANTHOCYANIDINS

Proanthocyanidins, which are also known as condensed tannins, are the oligomeric or polymeric forms of flavan-3-ols, or

flavanols, and are widely available in fruits, vegetables, and nuts. They are considered bioactive compounds because they influence physiological and cellular processes and therefore can have an effect on health.

The basic structural skeleton of proanthocyanidins contains two aromatic rings (A and B) connected by a pyrone ring (C). The benzenoid B ring of flavan-3-ols is in the 2-position. The most studied proanthocyanidins are based on the flavan-3-ols (+)-catechin and (−)-epicatechin. Other important flavan-3-ols are (+)-gallocatechin, (−)-epigallocatechin, and (−)-epigallocatechin gallate. Proanthocyanidins can have a variety of structures depending on the degree of polymerization, hydroxylation patterns, and stereochemistry (De Bruyne et al., 1999).

As natural antioxidants, proanthocyanidins have a broad spectrum of biological, pharmacological, and chemoprotective properties against free radicals and oxidative stress. They are antibacterial, antiviral, anti-inflammatory, antiallergic, and vasodilatory (Pinent et al., 2006); they inhibit lipid peroxidation, platelet aggregation, and capillary permeability and fragility, and they have hypolipidemic and cardioprotective properties (Roig et al., 2002; Puiggròs et al., 2005; Pinent et al., 2006; Terra et al., 2007; Aron and Kennedy, 2008; Ghosh and Scheepens, 2009; Serrano et al., 2009; Terra et al., 2009; Bladé et al., 2010).

In addition to their free radical-scavenging and antioxidant properties, proanthocyanidins have shown promising chemopreventive effects in various cell culture and animal models and also enhance the growth and viability of normal cells (Ye et al., 1999; Singletary and Meline, 2001; Agarwal et al., 2002; Surh, 2003; Tyagi et al., 2003; Kim et al., 2004; Singh et al., 2004).

7. PROANTHOCYANIDIN-INDUCED APOPTOSIS

The promising health benefits of the ability of proanthocyanidins to bimodulate apoptosis make consumption of this family of flavonoids a useful dietary strategy for preventing chronic pathologies. Like many drugs and chemopreventive agents, proanthocyanidins induce apoptosis through intrinsic or extrinsic pathways that inhibit or block carcinogenesis (Aparicio-Fernández et al., 2006).

Intrinsic Pathway

At high doses, proanthocyanidins are able to induce ROS formation in in-vitro carcinoma models, such as prostate PC-3 or DU-145 cells, thereby disrupting $\Delta\psi$m in a dose- and time-dependent manner. As shown in Table 1, the disruption of proanthocyanidin-induced mitochondrial outer membrane permeabilization (MOMP) leads to a PS reversion and the release of proapoptotic factors from the inner mitochondrial membrane (IMM), including cytochrome C, endonuclease G, Smac/DIABLO, and AIF, into the cytosol (Agarwal et al., 2002; Shang et al., 2009).

Proanthocyanidins, such as B2-3,3′-di-O-gallate (B2-G2) and B2-3′-O-gallate (B2-3′G), which are two proanthocyanidin B2 gallate esters isolated from grape seed proanthocyanidin (GSP) extract, or proanthocyanidins from the seed coats of black *Jamapa* beans, both result in the cleavage of the executor caspases-9, -7, and -3 in a concentration-dependent manner in DU145 and HeLa in-vitro models, respectively. Moreover, proanthocyanidins cleave PARP, a downstream substrate of activated caspase-3, which leads to typical apoptotic morphological features, such as chromatin condensation and DNA laddering (Agarwal et al., 2002; Aparicio-Fernández et al., 2008).

The integrity of the MOMP is controlled by the Bcl-2 (B-cell lymphoma-2) family, whose members are important regulators of the pathway. Bcl-2 and Bcl-xL are antiapoptotic proteins that control mitochondrial permeability and inhibit proapoptotic proteins, such as Bax, Bak, and Bad. However, proapoptotic proteins of the Bcl-2 family initiate apoptosis by blocking the mitochondrial binding sites and triggering the permeabilization of the mitochondrial membrane (Chou et al., 2010).

Proanthocyanidins have also been reported to have chemopreventive properties via the intrinsic pathway in vitro in breast cancer cells 4T1, MCF-7, and MDA-MB-468 by enhancing the expression of Bax. Other proanthocyanidins have demonstrated the same chemopreventive effects via caspase-3 activation in colon, leukemia, and oral cancer cell lines (Hong and Yi-Min, 2006; Mantena et al., 2006). B2-G2 and B2-3′-O-gallate downregulate the expression of Bcl-2 and Bcl-xl in prostate LNCaP cells and have no effect on the proapoptotic members Bax and Bad, which suggests an influence of proanthocyanidin gallate esterification because B2 proanthocyanidins do not have this effect (Agarwal et al., 2002; Chou et al., 2010). In contrast, proanthocyanidins from *Jamapa* bean increase Bax expression before the release of mitochondrial cytochrome C in human cervix adenocarcinoma cells (HeLa) (Aparicio-Fernández et al., 2006).

Apple proanthocyanidins and GSP increase mitochondrial membrane permeability and cytochrome C release from mitochondria, activate caspase-3 and caspase-9 in tumor cells in vitro, and likely increase the Bax:Bcl-2 ratio in vivo (Ray et al., 2005; Mantena et al., 2006; Miura et al., 2008).

As shown in Table 2, proanthocyanidin administration in several in-vivo models inhibited tumor growth and metastasis, and increased the survival in animal models. The proapoptotic effect is likely due to an increase in the Bax:Bcl-2 ratio (Nomoto et al., 2004; Mantena et al., 2006; Miura et al., 2008) or the inhibition of Bcl-xL antiapoptotic gene expression and its family members. Proanthocyanidin chemointervention in vivo has also been reported to induce DNA fragmentation, a hallmark of apoptosis (Ray et al., 2005), and to reduce proliferating cell nuclear antigen (PCNA), which is an index of cell proliferation (Nomoto et al., 2004) (see Table 1).

Remarkably, administration of apple proanthocyanidins in vivo induced apoptosis in tumor cells, whereas monomeric apple

Table 1 Modulation of apoptosis by proanthocyanidins in in-vitro models

Source	Disease	In-vitro model	Dose	Mechanism	Authors
Proanhocyanidins of black Jamapa beans	Cervical cancer Cancer Cervical cancer	HeLa - human adenocarcinoma HaCaT - human keratinocytes HeLa - human adenocarcinoma	35 μg/mL	Decrease in cells in the G0/G1 phase with no effect on the G2/M phase. Identification of DNA breakage. Increase in Bax and Caspase-3 expression.	Aparicio-Fernández et al., 2006 Aparicio-Fernández et al., 2008
Lotus seedpod proanthocyanidins	Skin cancer	B16 - mouse melanoma	50–100 μg/mL	Appearance of chromatin condensation and apoptotic bodies. Accumulation of cells in S and sub-G1 population. Stimulation of antioxidant enzymes activity.	Singh et al., 2004
B2-3,3′-di-O-gallate	Prostate cancer	DU145 - human prostate carcinoma	100 μg/mL	Increase in apoptosis via the intrinsic pathway.	Agarwal et al., 2002
Prodelphinidin B-2,3′-O-gallate from green tea leaf	Lung cancer	A549 - human nonsmall lung cancer	20 μM	Increase in nucleosome levels in the cytoplasm. Arrest of cell cycle in the G0/G1 phase phase via p53-independent induction of p21/WAF1. Induction of extrinsic pathway (Fas/FasL) and caspase-8.	Pierini et al., 2008
Apple proanthocyanidins	Esophageal cancer	OE-33 and OE-19 - human esophageal adenocarcinoma	60 μg/mL	Arrest of the cell cycle in G0/G1 and apoptosis. Effect correlated with degree of polymerization.	Lizarraga et al., 2007
	Mammary tumor	B16 - mouse melanoma andBALB-MC.E12 mouse mammary tumor	25 μg/mL	Increase in MMP and cytochrome C release. Activation of caspase-3 and caspase-9. Apoptosis maintained even with a caspase-8 inhibitor but not with a caspase-9 inhibitor, reinforcing the intrinsic pathway.	Miura et al., 2008
Cranberry proanthocyanidins	Ovarian cancer	SKOV-3 - ovarian cancer	200 μg/mL	Reduction of cell proliferation. Appearance of fragmented nuclei, condensed chromatin and apoptotic bodies.	Maldonado-Celis et al., 2009a
Proanthocyanidin (>98%)	Prostate cancer	PC3 - prostate carcinoma	300 μg/mL	Modulation of $\Delta\psi$m.	(Agarwal et al., 2002)
Proanthocyanidins from grape/pine bark	Colon cancer	HT29 human colon cancer	55 μM (grape) 123–127 μM (pine bark)	Inhibition of cell proliferation associated with antioxidant mechanisms. DNA condensation–fragmentation. Arrest of cell cycle in the G2 phase. Effects correlated with the galloylation index and mean degree of polymerization.	Lizarraga et al., 2007
Low oligomeric proanthocyanidins	Breast cancer	MCF-7 and MDA-MB 231	25 mg/mL	Modulation of the proapoptotic Bcl-2 family of proteins and the MEK/ERK signaling pathway.	Ma et al., 2010
Grape seed proanthocyanidin	Breast cancer	4T1 - breast cancer cells	40–60 μg/mL	Enhancement of Bax expression and increase in Bax:Bcl-2 ratio. Activation of caspase-3. Cleavage of PARP.	Mantena et al., 2006

(Continued on next page)

Table 1 Modulation of apoptosis by proanthocyanidins in in-vitro models *(Continued)*

Source	Disease	In-vitro model	Dose	Mechanism	Authors
Grape seed proanthocyanidins	Skin cancer	A431 - human epidermoid carcinoma	20–80 μg/mL	Inhibition of cell proliferation. Increase in G1-phase arrest via inhibition of cyclin-dependent kinases. (Cdks 2,4,6) and cyclins D1, D2, and E. Increase in protein expression of Cdki, Cip1/p21, and Kip1/p27. Increase in Bax expression and decrease in Bcl-2 and Bcl-xl. Loss of mitochondrial membrane potential. Cleavage of caspase-9, caspase-3, and PARP. Caspase inhibitor (z-VAD-fmk) blocks apoptosis.	Meeran and Katiyar, 2007
Apple proanthocyanidins	Colon cancer	SW480 - human colon adenocarcinoma	80 μg/mL	Upregulation of DR4, DR5. Apoptosis via extrinsic pathway.	Maldonado-Celis et al., 2009b
Grape seed proanthocyanidins	Colorectal cancer	HT29, SW-480, LoVo human colon carcinoma	12,5–50 μg/mL	Dose-dependent inhibition of cell proliferation. Activation of executor caspase-3 and cleavage fragment of PARP. Bcl-2 levels not affected.	Sherr, 2004; Kaur et al., 2006
	Acute myeloid leukemia	AML 14.3D10 - human myeloid leukemia cell	50 μg/mL	Integration between intrinsic and extrinsic pathways. Activation of caspases-2, -3, -8, and -9. Dose–response loss of $\Delta\Psi$m.	Hong and Yi-Min, 2006
Proanthocyanidins	Prostate cancer	LNCaP (androgen-responsive cells) PC3 (androgen-negative cells)	40 μg/mL	Decrease in the percentage of cells in the S phase and increase in sub-G1 phase. Inhibition of CDK and cyclins, and stimulation of tumor suppressors p21 and p27. Favorable changes in the Bcl-2/Bax ratio. Increase in phosphorylated MAPK p44 and p42.	Chou et al., 2010
Proanthocyanidins-enriched areca nut extract	Food allergy, autoimmune encephalomyelitis, inflammatory bowel disease.	Splenic lymphocytes obtained from male BALB/c mice.	20–60 μg/mL	Concentration-dependent decrease in the fraction of cells in the G0/G1 phase. Small, condensed nuclei. Inhibitors for caspase-6 and -3 (Z-VEID-FNK, and Z-DEVD-FMK, respectively) almost completely reverse apoptosis. Apoptosis associated with a decrease in the level of intracellular thiols, which was reverted by NAC.	Mandel and Youdim, 2004
Proanthocyanidins from cranberry	Prostate cancer	DU145 - human prostate carcinoma	25 μg/mL	Decreased expression of PI-3 kinase and AKT proteins and increased the phosphorylation of both p38 and ERK1/2.	Déziel et al., 2010

Table 2 Modulation of apoptosis by proanthocyanidins in in-vivo models

Source	Disease	In-vivo model	Dose	Mechanism	Authors
Grape seed proanthocyanidins	Colorectal cancer (CRC)	Fischer 344 rats AOM-induced aberrant crypt foci	0.25–0.5% (w/w)	Inhibition of AOM-induced cell proliferation. Decrease in PCNA-positive cells. Increase in apoptotic cells. Sharp decrease in cyclin D1, COX-2, iNOS, and survivin levels. Decrease in β-catenin and NF-kB expression levels in colon tissues.	Velmurugan et al., 2010a
			6.25–50 μM	Induction of apoptosis. Significant decrease in PCNA. Increase in single-strand DNA and nuclear fragmentation. Increase in hypodiploid cells in the sub-G1 phase. Increase in caspase-3.	Nomoto et al., 2004
	Skin tumor	SKH-I hairless mice UVB irradiation	0.25–0.5% (w/w)	Inhibition of UVB-induced infiltration of proinflammatory leukocytes. Inhibition of MPO, COX-2, PGE2, cyclin D1, and PCNA in skin tumors. Inhibition of proinflammatory cytokines, tumor necrosis factor-α, IL-1β, and IL-6.	Sharma and Katiyar, 2010
	Liver tumor	B6C3F1 mice Dimethylnitrosamine (DMN)	Co-and postadministration DMN + GSP (100 mg/kg bw)	Apoptogenicity efficiently prevented by antiendonucleolytic activity, which prevents cell death before tumor development and leads to an increase in Bcl-xL expression and a reversal of alkylating-toxin induced Bcl-xL phosphorylation. Apoptogenicity is efficiently intercepted after proanthocyanidin exposure by caspase activation and induces laddering of genomic DNA.	Ray et al., 2005
	Breast cancer	BALB/c mice with 4T1 tumor cells subcutaneously implanted	0.5%w/w	Increased ratio of Bax:Bcl-2. Increase in cytochrome C release, induction of Apaf-1, and activation of caspase-3.	Mantena et al., 2006
Lotus seedpod proanthocyanidins	Skin cancer	C57BL/6J mice	120 mg/kg bw	Prevention of apoptosis and tumor growth. Decrease in LPO and stimulation of AOS	Singh et al., 2004

polyphenols (AP) had no significant effect on the proliferation of either B16 or BALB-MC.E12 cells (Miura et al., 2008).

Bioavailability and Proanthocyanidin Structure and Apoptosis Through the Intrinsic Pathway

As with other dietary biofactors, the biological activities of proanthocyanidins are strongly dependent upon their stereochemistry, structure, and degree of polymerization, although the precise effect of the degree of polymerization remains unclear (Miura et al., 2008).

In fact, proanthocyanidin degree of polymerization of a particular plant or food depends on the natural source (e.g., grape, apple, fresh cocoa beans, or pears) and on the moment at which it is consumed. For example, the degree of polymerization in grapes varies between 9.8 and 31.5 and between 4.8 and 22.1 in red wines, indicating that there are substantial changes in flavan-3-ol composition during the fermentation and ageing of wines. A similar difference is observed between fresh cocoa beans and commercial cocoas and in tea products (Guyot et al., 1998; Ferreira et al., 2002; Crozier et al., 2009).

The degree of polymerization is important because it affects the bioavailability of proanthocyanidins. Even though proanthocyanidins are probably not absorbed in the small intestine when they are ingested, they are metabolized by the bacterial flora in the colon, and their metabolites may be able to explain some of the health-related effects that have been observed for proanthocyanidins.

Proanthocyanidins are believed to exert their biological effects in different ways: as unabsorbable, complex structures with binding properties that can have local effects in the gastrointestinal tract, as absorbable proanthocyanidins (probably low-molecular-weight), and as absorbable metabolites from the colonic fermentation of proanthocyanidins, which may have systemic effects in various organs (Serrano et al., 2009). Although long proanthocyanidins are absorbed less efficiently than short proanthocyanidins in the small intestine, they may have important local functions in the gut (Mota et al., 2009) as neutralizing oxidants and chemopreventive compounds (Gulgun et al., 2009). In experimental animals, various combinations of methylated, glucuronidated, and sulphate derivatives of flavan-3-ols, as well as native monomers, dimers, and trimers, have been detected in bodily fluids and tissues after the ingestion of proanthocyanidins (Baba et al., 2002; Tanaka et al., 2003; Tsang et al., 2005; Prasain et al., 2009; Urpi-Sarda et al., 2009).

In this regard, proanthocyanidins with an average degree of polymerization between 5 and 10 (PA5/10) reduce proliferation and induce apoptosis in rat colon cancer RCN-9 cells via the activation of caspase-3, whereas both monomers and proanthocyanidins with a degree of polymerization between 2 and 4 have a less pronounced effect. In addition, PA5/10 induces nuclear fragmentation, caspase-3 and -7 activation, and cell cycle arrest in the sub-G1 phase (Nomoto et al., 2004; Gossé et al., 2005), which is consistent with the reported cytotoxic effects of proanthocyanidins on MCF-7, A-427, and other carcinoma cell lines in vitro (Kozikowski et al., 2003; Gossé et al., 2005; Miura et al., 2008). Therefore, the way in which apoptosis is induced by the activity of pentamers and higher-degree procyanidins is of particular interest because the bioavailability of procyanidins has not been sufficiently investigated, and it is unclear whether procyanidin pentamers can be absorbed and transferred to tumor tissue.

Whereas GSP are galloylated to some extent, pine bark proanthocyanidins appear to be devoid of gallate esters (Rohdewald, 2002). In addition to the difference in bioavailability, GSP are reported to be more powerful antioxidants than pine proanthocyanidins, and GSP exhibit better antiproliferative and apoptotic effects. These data suggest that galloylation plays a more important role than polymerization in inducing apoptosis, and the characteristic matrix metalloproteinases (MMP) alterations, chromatin condensation, DNA fragmentation, and G2-phase cell cycle arrest are all more pronounced upon addition of GSP.

Extrinsic and Intrinsic Pathway

Proanthocyanidins from *Pinus massoniana* bark and prodelphinidin B-2 30-O-gallate enhance the extrinsic pathway. The former decrease Bcl-2 expression and phosphorylate the intact Bid protein, triggering caspase-8 activation and finally caspase-3 in Hep G2 cells, suggesting a cross-talk effect between the extrinsic and the intrinsic pathway. In contrast, the latter enhance the expression of Fas/FasL in A549 cells and reduce cell proliferation, blocking the cell cycle at sub-G1 and G2/M phases due to p53-independent induction of p21/WAF1. The extrinsic pathway is likely being targeted because no effects have been reported in the presence of antagonistic anti-Fas antibody or caspase-8 inhibitor (Lizarraga et al., 2007; Pierini et al., 2008; Ma et al., 2010).

Cell Cycle and Cell Signaling

As stated above, alternative cell signaling mitogenic pathways can be involved in apoptosis triggered by proanthocyanidins (Agarwal et al., 2002). Table 1 shows that proanthocyanidins have anticancer effects in several in-vitro carcinoma models because they modulate Bcl-2 family members and the MEK/ERK and JNK signaling pathways and affect cell cycle progression (Agarwal et al., 2000; Tyagi et al., 2003; Hudson et al., 2007; Meeran and Katiyar, 2007; Gao et al., 2009; Ma et al., 2010).

Therefore, proanthocyanidin extract from grape seed might exert its beneficial effects by means of increased apoptosis and suppression of the important PI3-kinase survival-related pathway, as shown in the attenuation of p110 and p85 subunits and decreased PKB Ser473 phosphorylation in colon cancer cells (Caco2) (Engelbrecht et al., 2007) or decreased expression of

PI-3 kinase and AKT proteins and increased phosphorylation of both p38 and ERK1/2 (Déziel et al., 2010).

It seems that proanthocyanidins increase ERK1/2 phosphorylation but do not contribute to a biological response because they have a direct inhibitory effect on the kinase activity of ERK1/2 for Elk1. In contrast, proanthocyanidins increase JNK1/2 activation, which leads to a strong apoptotic death (Tyagi et al., 2003). The involvement of JNK and its apoptotic effect is consistent with the interruption of the proanthocyanidin-mediated intrinsic pathway in the presence of SP600125, a JNK inhibitor (Gao et al., 2009).

Cancer manifests as the uncontrolled regulation of cell cycle progression; that is, members of the cyclin-dependent kinase inhibitor (CDKI) family are often nonfunctional, while cyclin-dependent kinases and the Cip/kip family are overexpressed (Kaur et al., 2006). Poanthocyanidins inhibit cell proliferation by interacting with the CDKI family and blocking cell cycle progression (Arbel-Goren et al., 2005; Morioka et al., 2005; Bloom and Cross, 2007; Besson et al., 2008; Kaur et al., 2008). Consistent with this, GSP-mediated apoptosis in Jurkat cells may be associated with Cip/p21 upregulation and cell cycle arrest through the activation of JNK in human leukemia cells and inactivation of JNK in the presence of SP600125 (Morioka et al., 2005).

At a molecular level, the suppression of β-catenin and NF-kB signaling with the downregulation of inducible isoform of nitric oxide synthase (iNOS), COX-2, cyclin D1, and survivin expression by GSP may revert inflammatory reactions, lead to apoptosis in damaged cells through the intrinsic pathway, and inhibit the early stages of chemical carcinogen-induced colonic aberrant crypt foci (ACF) formation and development by GSP in vivo (Morioka et al., 2005; Murakami, 2009; Velmurugan et al., 2010a, 2010b). Therefore, suppressing of the synthesis and/or activity of iNOS and COX-2 inhibits the MAPK and IkB kinases and their downstream transcription factors AP-1 and NF-kB, thus reverting inflammatory conditions by reducing proinflammatory cytokines and contributing to the anticarcinogenic effect of GSP (Tron et al., 1988; Bagchi et al., 2001).

p53 and the Apoptotic Effect of Proanthocyanidins

GSP protects against changes induced by oxidative stress and induces apoptosis by means of a p53-dependent mechanism in which the activation of Bcl-2, Bax, and caspase 3 are involved (Joshi et al., 2001; Gossé et al., 2006).

p53 is a tumor suppressor gene and orchestrates a global transcriptional response that either counters cell proliferation or induces apoptosis (Kim et al., 2004; Sherr, 2004). p53 enhances transcriptional activation of proapoptotic Bax and Bcl-Xs genes, thus modulating the Bcl-2:Bax ratio, altering MOMP, and triggering the intrinsic pathway (Joshi et al., 2001; Aruoma et al., 2003; Sherr, 2004; Roy et al., 2005; Aruoma et al., 2006).

It has also been proposed that GSP can modulate p53 gene expression (Aruoma et al., 2003) and oppose acetaminophen-induced phosphorylation of Bcl-XL in the liver in vivo (Mandel and Youdim, 2004).

8. APOPTOSIS AND OXIDATIVE STRESS

Oxidative stress has been associated with some chronic pathologies and metabolic disturbances, and some chemotherapeutic agents and radiation generate ROS in patients during cancer therapy. Many chemopreventive agents with antioxidant activity can modulate genes or proteins that respond to conditions of oxidative stress and apoptosis through mitochondrial alterations and MAPK signaling pathways. Phytochemicals may also quench ROS involved in apoptosis, thus acting as anticarcinogenic, or cardiovascular and neuroprotective agents, depending on the extracellular stimulus and cellular homeostatic environment (Kluck et al., 1997; Mattson and Kroemer, 2003; Scalbert et al., 2005), such as the enzyme antioxidant system or glutathione (GSH) levels (Li et al., 2006).

Apoptosis occurs in the human heart during the end stage of cardiac failure, and it is well known that proanthocyanidins protect against ROS-mediated myocardial ischemia/reperfusion injury and apoptosis of cardiomyocytes (Pataki et al., 2002). However, whereas proanthocyanidins have become increasingly popular for promoting health and preventing disease, concerns have been raised about GSP toxicity at high doses (So et al., 1996; Skibola and Smith, 2000; Shao et al., 2003b; Shang et al., 2009; Chou et al., 2010). In fact, several reports have demonstrated that high doses of proanthocyanidins may cause cytotoxicity associated with caspase activation and increased apoptotic cell death (Shao et al., 2006) probably because activation of iNOS leads to an overproduction of NO and a depletion of intracellular GSH (Wouters et al., 2005).

Moreover, anticancer drugs such as doxorybicin can evoke cardiotoxicity due to the formation of ROS. This potential cytotoxicity compromises the clinical usefulness of these drugs (Horenstein et al., 2000) and ultimately results in mitochondrial dysfunction and myocyte apoptosis (Du and Lou, 2008; Sardao et al., 2008). Proanthocyanidins significantly inhibit doxorubicin-induced intracellular ROS accumulation, prevent DNA fragmentation, and inhibit apoptosis by modulating Bcl-2 family proteins (Du and Lou, 2008). Similarly, proanthocyanidins reduce ROS accumulation and cardiac cell apoptosis induced by xanthine oxidase (XO)/xanthine-induced system (Du et al., 2007) by activating a coordinated network of the antioxidant enzyme system combined with a proapoptotic signal in damaged cells (Shao et al., 2003a).

Several diseases associated with senescence appear to be the direct result of cells containing dysfunctional mitochondria. Because mutated mitochondrial DNA (mtDNA) can impair energy conversion and increase ROS production, it has been associated with progeriatric syndromes such as Alzheimer's disease, diabetes, degeneration, and several progressive muscle diseases, as well as the ageing process itself (Aruoma et al., 2006)

Table 3 Proanthocyanidin antiapoptotic effects and mechanism involved

Source	Disease	In-vitro model	Dose	Mechanism	Authors
Cocoa proanthocyanidins A and B2 (epicatechin-(4β-epicatechin)	Alzheimer	PC12 rat pheochromocytoma Apotosis induced by H_2O_2	1–5–10 μM	Proanthocyanidins pretreatment avoids PARP cleavage by H_2O_2 exposure. Increase in antiapoptotic Bcl-XL and Bcl-2 expression. Inhibition of caspase-3 and attenuation of JNK and p38 mitogen MAPK.	Feng et al., 2007
Cocoa proanthocyanidins	Alzheimer	PC12 rat pheochromocytoma induced to apoptosis with 4-hydroxinonenal	5–10 μg/mL	Inhibition of HNE-induced nuclear condensation. Inhibition of cell cycle progression arrest by inhibition of the sub-G1 fraction accumulation. Attenuation of ROS formation. Prevention of HNE-induced PARP cleavage, antiapoptotic protein (Bcl-2 and Bcl-XL) downregulation and caspase-3 activation. Attenuation of activation of JNK and MKK4.	Feng et al., 2007
Proanthocyanidin B4 from grape seed	Cardiovascular diseases	Rat heart cell line H92C2 exposed to XO (xanthine oxidase)	100 μM	Protection against apoptotic changes in nuclear morphology, cell shrinkage, blebbing, and chromatin condensation. Induction of endogenous antioxidant system (SOD, CAT, GSH-Px, and GST) activities. Inhibition of [GSH] decrease.	Du et al., 2007
		Rat heart cell line H92C2 with doxorubicin treatment		Decrease in ROS generation. Decrease in the number of apoptotic cells and prevention of DNA fragmentation. Reduction of the Bax/Bcl-2 ratio, thus inhibiting apoptotic signaling pathways.	Du and Lou, 2008
Cinnamtanin-B1	Platelet alteration	Thrombin-activated platelets	10 μM	Reduction of thrombin-evoked phosphatidylserine (PS) externalization. Reduction of caspase-3 and -9 activation and impairment of their cytoskeleton translocation.	Bouaziz et al., 2007
Grape seed proanthocyanidins	Alzheimer	PC12 induced to apotosis with β-amyloid	25 μM	Prevention of DNA fragmentation. MOMP restoration and suppression of PARP cleavage. Amelioration of MDA formation and recovery of GSH levels. Inhibition of NF-κB signaling pathways (suppression of IκBα cytoplasmatic degradation and p65 translocation to the nucleus).	Li et al., 2004a

The potential neuroprotective effects of phenols on the neuronal deficits associated with ageing or age-related neurodegenerative diseases are also of increasing interest (Aruoma et al., 2003; Steinberg et al., 2003; Li et al., 2004b; Zbarsky et al., 2005; Sutherland et al., 2006; Zaveri, 2006).

Oxidative stress-induced apoptosis via the intrinsic pathway in neurons has been associated with alterations in the Bcl-2 family and the participation of the MAPK family. In fact, ERK, JNK, and p38 MAPK are apoptotic factors mediated by oxidative stress, and abnormal levels of these proteins have been observed in the brains of patients with Alzheimer's disease (Zhu et al., 2002).

9. INACTIVATION OF APOPTOSIS BY PROANTHOCYANIDINS

Intrinsic Pathway

Proanthocyanidins from several natural sources reduce cell death caused by oxidative chemicals or stress agents and attenuate nucleus and DNA fragmentation mostly via the intrinsic pathway. That is, the antiapoptotic Bcl-2 family proteins are increased and the mitochondrial alterations are restored (see Table 3) (Joseph et al., 1999; Anuradha et al., 2001; Ono et al., 2004). As stated above, the antioxidant GSH protects against the loss of MOMP resulting from lipid peroxidation, and proanthocyanidins inhibit apoptotic events via the intrinsic pathway, along with restoring GSH levels in PC12 cells with amyloid β in vitro, which has been reported to induce NF-kB in neurons and astrocytes, and appears to be mediated by ROS formation (Ito et al., 2003; Bai and Cederbaum, 2006; Kenny et al., 2007). As described above, the strength of the apoptotic effect depends on the average degree of polymerization. Fractionated oligomeric proanthocyanidins from pentamers to decamers actively induce apoptosis, whereas monomers to tetramers do not (Tamago et al., 2003).

Proanthocyanidin antiapoptotic effects also involve other physiological mechanisms for remodeling tissues and organs. For example, platelets express several components of the apoptotic machinery present in nucleated cells, including caspases-3 and -9 and Apaf-1. This suggests that anucleated platelets undergo an apoptotic program and can be used as a physiological model of nucleus-independent cytoplasmic apoptosis. In fact, human platelets develop apoptotic features after agonist stimulation or under shear stress. Because platelet stimulation with thrombin induces endogenous H_2O_2 production and evokes apoptotic events in platelets, it has been suggested that endogenously generated H_2O_2 has a role in thrombin-induced apoptosis in these cells. Cinnamtannin B-1 has a clear antiapoptotic effect in human platelets, as demonstrated by its inhibitory effect on thrombin-evoked PS externalization and activation and translocation of caspases-3 and -9 to the cytoskeletal fraction (Baines and Molkentin, 2005).

Cell Cycle and Cell Signaling

Proanthocyanidins suppress ROS accumulation induced by oxidative stress and increase cell cycle progression by decreasing p53 mRNA and c-jun expression and blocking apoptosis (Bouaziz et al., 2007; Haddad, 2004), exhibiting both antioxidant and antiapoptotic effects through the c-jun pathway (Saporito et al., 2000).

As stated above, oxidative stress activates MAPK. Proanthocyanidins attenuate this effect, which plays a crucial role in preventing or reducing neuronal apoptosis (Sen and Packer, 1996; Buonocore et al., 2001; Xu et al., 2003). In this regard, proanthocanidins protect against oxidative stress-induced apoptosis by blocking the activation of JNK and p38 MAPK and inhibiting the downregulation of Bcl-XL and Bcl-2 expression (Duan et al., 2010).

Moreover, proanthocyanidins attenuate Aβ-induced degradation of cytoplasmic IkB kinase subunit, nuclear translocation of p65, and DNA binding of NF-kB in PC12 cells. Further studies are necessary to clarify the implications of NF-kB inactivation by proanthocyanidins in the protection of Aβ-induced apoptosis in PC12 cells (Li et al., 2004a).

CONCLUDING REMARKS

Apoptosis is a critical molecular target for dietary bioactive agents for the chemoprevention of cancer and other health benefits. It is a complex process that involves mostly intrinsic and extrinsic pathways, but it is encouraging that single bioactive dietary agents can directly and indirectly influence core targets within apoptosis.

Proanthocyanidins have numerous biological activities and should not be considered only as free-radical scavengers. They decrease tumor cell proliferation by enhancing apoptotic mechanisms, but they can also delay cellular decline and inhibit programmed cell death without compromising the function of normal cells.

Moreover, the protective effects of some single agents are potentiated and/or synergized by other dietary agents, which supports the notion that a combinatorial approach will be especially effective in apoptosis modulation. However, many aspects still need to be elucidated such as the appropriate dose, structural and chemical considerations, and length of exposure.

Finally, as dietary components, proanthocyanidins can modulate gene expression and metabolism in vivo and contribute to numerous processes such as apoptosis and cell signaling. Individual polymorphisms are a challenge to research human responses to proanthocyanidins, and subsequent modulation of so many more studies following this approach are necessary to better characterize responses and the physiologic consequences of genetic polymorphisms and their implication on delaying, preventing, or treating chronic pathologies.

In summary, the dual or differential modulation of apoptosis by proanthocyanidins in healthy (antiapoptotic actions) and

tumor cells (proapoptotic effects) suggests that these compounds could be used to provide new and selective strategies for improving health and/or delaying chronic pathologies.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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ABBREVIATIONS

AIF = Apoptosis-inducing factor
Apaf-1 = Apoptotic protease activating factor-1
DIABLO = Direct IAP-binding protein with low pI
DISC = Death-inducing signaling complex
DR = Death receptor
ER = Endoplasmic reticulum
ERK = Extracellular signal regulated protein kinases
FADD = Fas-associated protein with death domain
GSH = Glutathione
GSP = Grape seed proanthocyanidins
IAP = Inhibitor of apoptosis proteins
IMM = Inner mitochondrial membrane
JNK = c-Jun N-terminal protein kinases
MAPK = Mitogen-activated protein kinases
MOMP = Mitochondrial outer membrane permeabilization
NF-κB = Nuclear factor κB
PARP = Poly (ADP-ribose) polymerase
PCNA = Proliferating cell nuclear antigen
PS = Phosphatidyl serine
ROS = Reactive oxygen species
Smac = Second mitochondria-derived activator of caspases
TNF = Tumor necrosis factor
TRADD = Tumor necrosis factor receptor type 1-associated death domain protein
TRAIL = TNF-related apoptosis-inducing ligand
TRAF = TNF receptor-associated factor

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