

Natural Polyphenols and Apoptosis Induction in Cancer Therapy

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Abstract

Natural polyphenols are secondary metabolites of plants involved in the defense against different types of stress. Due to their potent antioxidant properties, polyphenols have shown potential health benefits in various oxidative stress-associated diseases such as cancer. Polyphenols can induce tumor cell death and interfere with carcinogenesis, tumor growth, and dissemination. Understanding of tumor biology, identification of novel molecular targets, development of specific antitumor agents, and increase the susceptibility of tumor cells to conventional/targeted treatments are key points for the achievement of effective anticancer therapies.

This review focuses on the antitumor effect of four well known natural polyphenols targeting apoptosis pathways: (-)-Epigallocatechin-3-gallate, resveratrol, curcumin and pterostilbene. *In vitro* and *in vivo* studies involving effects and mechanisms of polyphenols-induced apoptosis are discussed, as well as different strategies to improve their bioavailability, which is a main problem, limiting their therapeutic use.

Keywords: Polyphenol; Cancer; Apoptosis

Abbreviations: Apaf-1: Apoptotic Protease Activating Factor-1; Cyt-c: Cytochrome-c; Bid: Bcl-2 Interacting Domain; DAPK1: Death Associated Protein kinase 1; DISC: Death Inducing Signaling Complex; EGCG: (-)-epigallocatechin-3-gallate; DR: Death Receptor; FADD: Fas-Associated via Death Domain; c-FLIP: FADD-like interleukin-1 β -converting enzyme Inhibitory Protein; Fas: Fatty Acid Synthetase; MDM2: Mouse Double Minute 2; PCD: Programmed Cell Death; ROS: Reactive Oxygen Species; STAT3: Signal Transducer and Activator of Transcription 3; TNFR: Tumor Necrosis Factor Receptor; TRAILR: TNF-Related Apoptosis-Inducing Ligand Receptor; TRADD: Tumor Necrosis Factor Receptor-1-Associated Death Domain

Survival through Cell Death Control

It can result paradoxical that stimulation of cell demise may facilitate organism survival. However a variety of physiological processes, such as germ cells control [1,2], morphogenesis [3-5], tissue homeostasis [6], etc. involve a tight regulation of cell death. During the past 25 years, Programmed Cell Death (PCD) type I or apoptosis has been extensively studied. Although, actually, this natural cell death process was already observed 170 years ago, and was considered a passive phenomenon linked to the end of functional biological systems [7,8]. Kerr, Wylie and Currie, 40 years ago, described the specific morphological and biochemical alterations of apoptotic cells under physiological conditions [9]. At present, two other (and different) forms of PCD have been accepted, autophagy or PCD type II and necrosis or PCD type III [10].

Apoptosis is derived from an ancient Greek word that seems to refer to "leaves falling from a tree" [11]. In contrast to the swelling of the cell and its organelles that defines necrosis, the main morphological feature of apoptosis is shrinkage of the cell and nuclear fragmentation [10]. Besides autophagy is a process in which cells generate energy by digesting their own organelles and macromolecules. Thereby autophagy allows a starving cell (or a cell deprived of growth factors) to survive. Ultimately, cells that do not receive nutrients for a prolonged period of time digest their own structures and die [12]. Consequently, the existence of alternative mechanisms leading to cell death plays a fundamental role in development, environmental adaptations, and survival.

According to accepted models, apoptotic cell death can result

from activation of two different but interrelated molecular cascades. An extrinsic pathway transduces the extracellular stimulus, protein death ligands, through plasmatic membrane. With our present knowledge the death receptor family in charge of starting this extrinsic pathway include: Tumor Necrosis Factor Receptor 1 (TNFR), Fatty Acid Synthetase (Fas) receptor, Death Receptor (DR) 3, TNF-Related Apoptosis-Inducing Ligand Receptor (TRAILR1/R2) or DR4/5 and DR6 [13]. Besides, an intrinsic pathway controls and monitors the intracellular environment translating this information into integration centers, the mitochondria, which evaluate the molecular signals leading to death or survival. Proapoptotic signals such as oxidative stress, damage in DNA, and alterations in mitochondrial membrane trigger apoptosis activation. These two pathways show a convergent point where caspases unify the control of the process.

Cell death resistance, as a causal phenomenon linked to abnormal regulation of the apoptotic process, is associated with several human pathologies such as developmental disorders, immune and degenerative diseases, cancer, etc [11]. In particular, the ability to induce PCD under control conditions has been one of the focuses for a large number of research groups. Indeed, the molecular pathways (extrinsic and intrinsic) regulating the apoptotic process are attractive targets for potential therapeutic intervention. In this sense, the development of agents capable of stimulating or inhibit the action of physiological proapoptotic or antiapoptotic molecules, respectively, has received a lot of support by the pharmaceutical industry. For instance, the goal of proapoptotic drugs is to selectively induce apoptosis in the tumor cell while leaving healthy cells unharmed. Several proapoptotic receptor agonists have recently been developed, activating selectively the extrinsic pathway, and give promising results. Targets for the intrinsic

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pathway include the Bcl-2 family proteins, the inhibitor of apoptosis proteins, the p53 pathway, and many others [14,15].

The large number of publications reflects the impressive research work done on cancer and apoptosis. In PubMed more than 100,000 papers can be found since the publication by Kerr JF et al. of an original manuscript describing apoptosis as a basic biological phenomenon [9]. Studies involving apoptosis control in cancer therapy have used different strategies concerning initiation, progression and/or invasion, the three main stages of carcinogenesis. However, the development of tumor resistance and the adverse side effects of conventional and target-oriented therapies imply the need of novel treatment strategies with lower toxicity.

Natural polyphenols, the most abundant antioxidants in human diet, have many potential benefits in human health [16]. The relationship between natural polyphenols, apoptosis and cancer was identified by studies on the ability of these compounds to act as cancer chemopreventive and/or chemotherapeutic agents [17-19]. Polyphenols, in addition to their antioxidant activity and among many potential mechanisms, e.g. regulate the expression of target genes involved in cell survival and proliferation [20], induce different programs of regulated cell death [21], or inhibit matrix metalloproteinases [22] and vascular endothelial growth factor [23], thus counteracting angiogenesis and affecting to metastasis development. The present review will focus on the molecular basis of apoptosis induction triggered by different key polyphenols, including resveratrol, curcumin, (-)-epigallocatechin-3-gallate (EGCG) and pterostilbene.

Overview of the Apoptotic Pathways in Cancer

The extrinsic pathway is activated when stimulus outside the cell in form of specific ligands bind to its corresponding cell death receptors located in plasma membrane surface. DRs belong to the TNFR superfamily, and are characterized by a Cys-rich extracellular domain and a homologous intracellular domain known as the death domain. Some examples of DRs are TNFR, TRAIL receptor, and Fas receptor (also called APO-1 or CD95) [24]. Moreover there are different adapter molecules with death domains, which permit the interaction with DRs, such as FADD (Fas-Associated via Death Domain) [13, 25] or TRADD (Tumor Necrosis Factor Receptor-1-Associated Death Domain) [25].

Fas receptor is activated by binding of the homotrimeric protein called Fas ligand, which causes oligomerization of its receptor and recruitment of FADD. This protein binds, via death domain motif, to a homologous motif on procaspase 8. The complex formed by Fas, FADD and procaspase-8 is known as the Death Inducing Signaling Complex (DISC) [10,26]. FADD-dependent caspase-8 activation may be blocked by FADD-like interleukin-1 β -converting enzyme Inhibitory Protein (c-FLIP) [27]. Procaspase-8 recruitment drives its activation through autocatalysis, whereas Caspase-8 activates downstream Caspase-3 and -7 [28]. Activated caspase-8 cleaves Caspase-3, directly and indirectly [29]. The indirect mechanism includes the activation of the Bcl-2 Interacting Domain (Bid), a proapoptotic protein of the Bcl-2 family members. Upon Bid cleavage, its carboxy-terminal part migrates to the mitochondria where triggers Cytochrome-c (Cyt-c) release from mitochondrial intermembrane space. Thus Bid, through its effect on the mitochondrial membrane potential, interacts with the intrinsic pathway [29]. In the cytosol Cyt-c, Apoptotic Protease Activating Factor-1 (Apaf-1), dATP, and procaspase-9 form a supramolecular complex called apoptosome that activates caspase-9 through self-cleavage [13,29]. Caspase-9 cleaves procaspase-3 activating Caspase-3

[10,13,30]. Therefore, both pathways lead activation of Caspase-3, which in turn activates other executor proteases (Figure 1).

The intrinsic apoptotic pathway involves via the mitochondria and is initiated by proapoptotic factors release. These factors either activate caspases or enhance caspase activity. The intrinsic or mitochondrial pathway may be activated by different causes such as e.g. Reactive Oxygen Species (ROS), toxics, drugs, ionizing radiations, etc. The activation of this pathway is accompanied of Cyt-c, translocation to the cytoplasm, apoptosome formation and caspase-9 autocatalysis [29] (Figure 1).

The Bcl-2 family members exert a critical regulatory role in determining cellular viability. This family includes the main checkpoints to control the evolution of the apoptotic process. In fact, the ratio between pro-apoptotic (Bax, Bad, Bak, Bid, Bcl-Xs) and anti-apoptotic (Bcl-2, Bcl-xL, Bag-1, Bcl-W) members is an indicative of the mitochondrial membrane potential status [31,32].

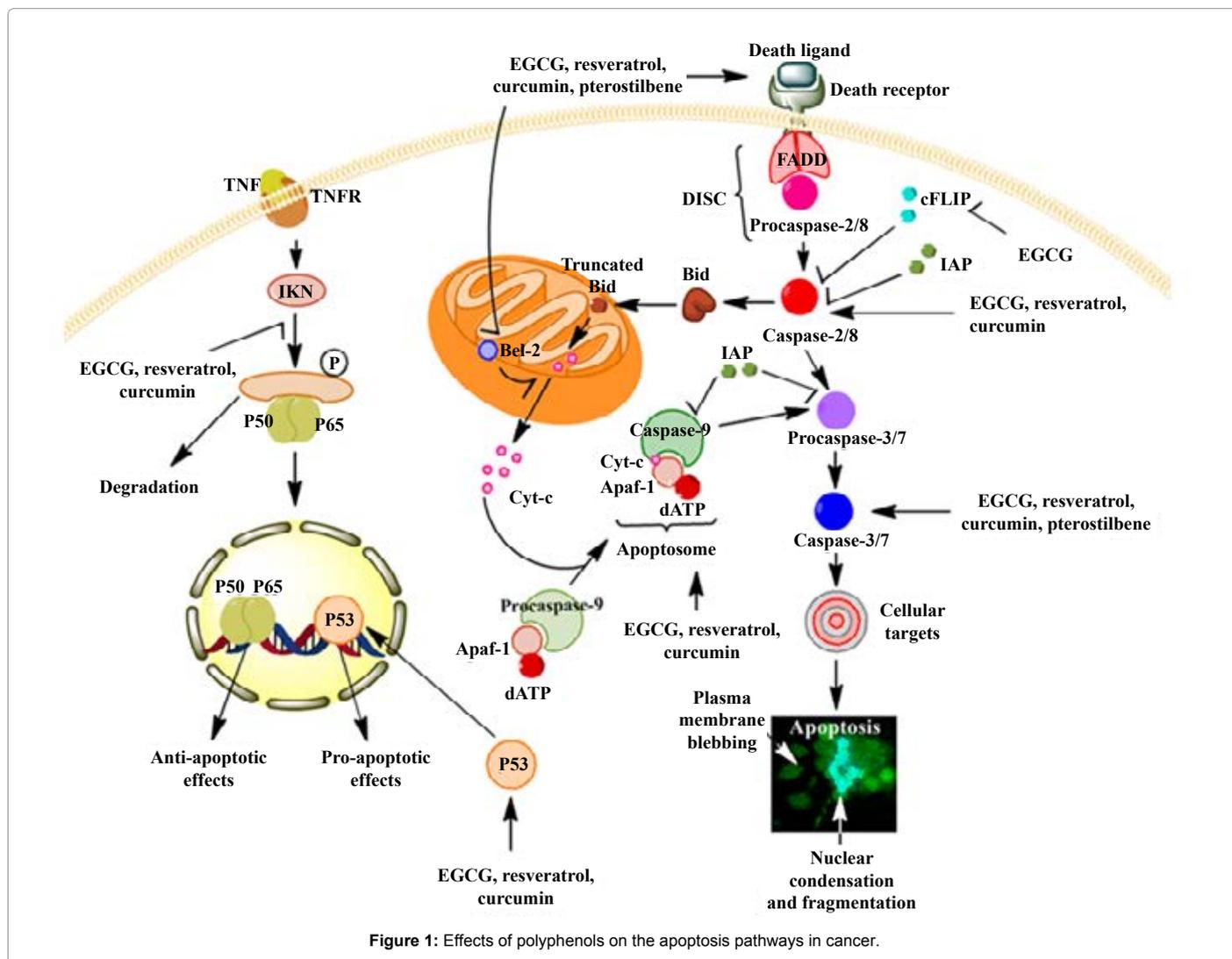
Other key molecule in apoptosis regulation is the transcription factor p53. The main role of p53 is the protection against genomic instability and tumorigenesis [33]. Functionally promotes survival by activating checkpoints and facilitating damage repair [33-35], sustained proliferation blocking [33,36] and apoptosis [33,35-37]. Tetramerization of p53 monomers is essential for its ability to positively regulate transcriptional activation of a large number of targets including its downstream effector p21^{WAF1}, and the proapoptotic proteins Bax, PUMA, and Noxa [37,38]. On the other hand, p53 negatively regulates the transcription of a large number of antiapoptotic genes such as Bcl-2 [39], Mcl-1 [40] and survivin [41].

The tumor suppressor P53 is involved in multiples cellular processes and in the response to a variety of stresses, including DNA damage. DNA-damaging agents and other stress stimuli stabilize and activate P53, present at low levels in resting cells, through post-translational modifications that release it from MDM2 (mouse double minute 2 but used interchangeably to denote human also), a ubiquitination ligase that ubiquitinates it prior to proteasome degradation [33].

NF κ B is a transcription factor associated with many physiological processes such as, inflammation, cellular proliferation and cancer [42]. NF κ B, a heterodimer composed of p50 and p65 subunits, is sequestered in the cytosol in an inactive form upon interaction with I- β -B [43]. Different factors can trigger its activation, i.e. free radical, tumor promoters, cytokines, UV light, endotoxins, etc. After stimuli, I- β -B is phosphorylated and removed by the proteasome, which facilitates NF κ B translocation into the cellular nucleus and its consequent activity as gene expression regulator [44].

Natural Polyphenols and their Benefits for Human Health

Natural polyphenols are a very large group of plant-derived compounds structurally characterized by the presence of two or more phenol units [45]. The most chemistry aware definition of the polyphenol term (known as the White-Bate-Smith-Swain-Haslam definition) describes the class as (i) generally moderately water-soluble compounds, (ii) with molecular weight of 500-4000 Da, (iii) >12 phenolic hydroxyl groups, and (iv) 5-7 aromatic rings per 1000 Da, where the limits to these ranges are necessarily somewhat flexible [46]. However, this type of definition does not include lower molecular weight structures, which have been shown to have potential benefits for human health. In practice, this implies a less rigorous use of the



polyphenol term toward the lower molecular weight end of the range. As a consequence, there are several thousand compounds in higher plants of potential biological interest with one or more aromatic rings and at least two hydroxyl groups, thus qualifying as polyphenols [47].

Natural polyphenols are secondary metabolites in plants which are produced as a defense against different types of stress, e.g. ultraviolet radiation, aggression of pathogens, low soil fertility, changes of environmental temperature, severe drought, and grazing pressure [45,48]. Depending on their basic chemical structure, four main classes are considered: phenolic acids, flavonoids, stilbenes, and lignans [48,49]. The knowledge and implications of these compounds in human health include beneficial effects in cancer [50-52], neuroprotection [53,54], cardiovascular system dysfunction and damage [47,55], the metabolic syndrome [56,57], diabetes [58], aging [59,60], and different inflammation-related pathologies [60]. Due to all these benefits, polyphenols have been used for thousands of years in traditional eastern medicine. Nevertheless incorporation of these compounds to western medicine is still a pending issue, likely because poor oral bioavailability strongly limits their potential effects [61]. In fact, and generally speaking, correlations between *in vitro* effects and *in vivo* findings are, at present, poorly established.

Natural Polyphenols and Apoptosis Targeting in Cancer Cells

Cancer can be viewed as a complex cellular phenotype which is associated with unlimited replicative potential, independence from growth signals and parallel resistance to growth-inhibitory signaling, evasion of cell death activation, sustained angiogenesis, as well as the ability of tissue invasion and metastasis [62]. Malignant tumors are invasive, and may metastasize to distant sites through the circulatory system. Consequently, metastatic spread, not the primary tumor burden, is the main cause of cancer-related deaths [63].

At present, strategies of cancer treatment using the combination of targeted therapies and established/conventional chemotherapies or radiotherapies are considered more promising, and may lead to greater efficacy and better survival [64]. Nevertheless, malignant cells may find alternative survival mechanisms. Indeed, changing conditions within tissue microenvironments, systemic/intra-organ signals, immune cells attack, or therapy-related cancer cell stress, may cause alterations in the genomic/proteomic profile of metastatic cells leading to either cancer suppression or survival. It is in this general scenario where natural polyphenols, which have been shown to interfere with carcinogenesis

[65,20], tumor growth [50] and dissemination [66,67], may be potentially useful. For instance, early reports have suggested that polyphenols have protective effects in the development of pulmonary neoplasia [68,69]. In addition, they are able to inhibit lung carcinoma cell proliferation and metastasis by different mechanisms [70-73]. It has been reported that tea polyphenols and atorvastatin synergistically inhibit chemically-induced murine lung tumorigenesis and the growth of lung cancer H1299 and H460 cells, possibly through enhanced apoptosis [74]. Another recent report shows that epicatechin enhances the induction of growth inhibition and apoptosis in human lung cancer cells by curcumin [75]. Thus, suggesting possible application in lung cancer therapy.

(-)-Epigallocatechin-3-gallate (EGCG), (abundant in green tea), curcumin (a major component of turmeric), and resveratrol (a phytoalexin found in grapes, red wine and mulberries), have been shown effective in the treatment of metastatic prostate cancer with therapeutic resistance. *In vitro* studies show that EGCG, curcumin or resveratrol sensitize LNCaP prostate cancer cells to TRAIL-mediated apoptosis through modulation of the extrinsic apoptotic pathway [76-78]. Moreover, EGCG reduces endogenous lipid synthesis, inhibits cell growth and induces apoptosis by inhibition of Fas in cells with high levels of Fas activity such as LNCaP (an effect not observed in non-tumoral cells) [79]. Furthermore, polyphenols can also induce apoptosis by intrinsic pathway. Resveratrol induces apoptosis through the intrinsic pathway. For instance, resveratrol induces apoptosis in prostate cancer-derived cell lines by activating caspases-9 and -3 and by changing the Bax/Bcl-2 ratio [80]; and similar effects were observed with EGCG, curcumin [76,81], or pterostilbene [82,83] in different cell types.

Recently, preclinical experiments using human HT-29 colorectal xenografts, have shown that combined administration of pterostilbene, quercetin, FOLFOX6 (oxaliplatin, leucovorin, and 5-fluorouracil; a first-line chemotherapy regimen), and radiotherapy eliminates growing tumors *in vivo* leading to long-term survival (>120 days). Antisense oligodeoxynucleotides against human superoxide dismutase 2 and/or ectopic Bcl-2 overexpression avoided polyphenols- and chemoradiotherapy-induced colorectal cancer elimination; thus suggesting SOD2 and Bcl-2 as key targets in the mechanism [84]. Furthermore, some early reports suggested that oral administration of green tea polyphenols, even at low doses, was effective in preventing chemically induced colon carcinogenesis by inhibiting angiogenesis and metastasis, and by inducing growth arrest and apoptosis through regulation of multiple signaling pathways [85,86]. These results suggest that administration of polyphenols may facilitate colorectal cancer regression *in vivo*.

Therefore, administered alone, in combination with conventional chemoradiotherapy, or with other polyphenols (using topic, oral, intraperitoneal injection, or intravenous administration), polyphenols appear active to prevent the appearance and spread of cancer. Moreover, in this anticancer activity, induction of apoptosis appears to play a relevant role. Due to the complexity of the effects induced by each particular polyphenol our subsequent analysis will focus on some specific polyphenols, all particularly relevant for their potential clinical applications. Recently, our group has published a review on natural polyphenols and their application in oncotherapy, where clinical trials involving 3 of those specific polyphenols mentioned (resveratrol, curcumin, and epigallocatechin-3-gallate) have been discussed [87].

Due to the large number of polyphenols with potential anticancer properties, we have focused only on examples involving active clinical

trials according to the US NCI (<http://www.cancer.gov/clinicaltrials>). Nevertheless, reported effects claimed for polyphenols must be carefully evaluated as one finds differences (even large and controversial) depending on the type of cancer cell, the experimental *in vitro* or *in vivo* conditions, the concentrations/galenic formulations/nanoparticle associations used.

Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene), a naturally occurring phytoalexin produced by plants in response to damage, possess important antioxidant, anticarcinogenic, and antitumor properties. It was first detected in dried roots of *Polygonum cuspidatum*. It is a member of the stilbene family and can be found in isoforms *cis*- and *trans*-, being *trans*-resveratrol is the more abundant natural isoform [88, 89]. The ability to induce carcinogenic inhibition was reported in 1997 by Jang et al. [19]. Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes (anti-initiation activity); it mediated anti-inflammatory effects and inhibited cyclooxygenase and hydroperoxidase functions (antipromotion activity); and it induced human promyelocytic leukemia cell differentiation (antiprogession activity). In addition, it inhibited the development of preneoplastic lesions in carcinogen-treated mouse mammary glands in culture and inhibited tumorigenesis in a mouse skin cancer model [19]. Although, nowadays, the antitumor effects of resveratrol are still incompletely understood, there are numerous evidences in different types of cancer (melanoma, prostate, leukemia, colon, breast, lung...) that show the capability of this polyphenol to induce apoptosis [90-96]. The proapoptotic stimulation has been associated to cell cycle alterations [95,97], caspase activity induction [92,95, 97,98], downregulation of Bcl-2, Bcl-xL, Survivin and XIAP levels [99], upregulation of Bax levels [98, 99], Bak, PUMA, Noxa, Bim, TRAIL-R1/DR4 and TRAIL-R2/DR5 [101]. Interestingly, a number of these effects may be correlated with P53 activation [92,97-98].

The stabilization and activation of P53 depends on both acetylation and phosphorylation modifications that determine the binding to DNA. Phosphorylation of P53 prevents the binding with MDM2 [33] and facilitates the acetylation of P53 at a C-terminal lysine [100]. P53 acetylation increase sequence-specific DNA binding and recruits coactivators/histone acetyltransferases such as CREB-binding protein/p300 [101]. Resveratrol induces P53 phosphorylation in N terminal serine 15 (Ser-15) [102,103] and C-terminal serine 392 (Ser-392) [102], and acetylation [104] are induced. Moreover, treatment of a mutant P53 prostate cancer DU145 cells with resveratrol induced phosphorylation of Ser-15 which restored wild-type P53 DNA binding [103,105] and P53 acetylation [104], activating pro-apoptotic events. In addition, it has been shown, in MCF7 breast carcinoma cells, that resveratrol also phosphorylates Ser-15 and induces expression of various P53-regulated proapoptotic proteins (p21, Bax, and Fas), caspase 8/9 activation, and decreases Bcl-2 expression [105].

It has been postulated that some resveratrol-induced anticancer effects are due to its capacity to inactivate NFκB -dependent signaling. Resveratrol inhibits IKK mediated IβB phosphorylation by stimulating the retention of NFκB in the cytosol and its subsequent inactivation [106,107]. Furthermore, resveratrol inhibits proliferation, causes cell cycle arrest, suppresses NFκB-dependent signals related to proliferation and survival [108, 109], and blocks the activation of NFκB induced by heme oxygenase 1, IL-1α and TNFβ [108,110,111].

(-)-Epigallocatechin-3-gallate

Green tea (*Camelia sinensis*) is a popular beverage being, after water,

the most habitual liquid consumed worldwide. The main antioxidative ingredients in the green tea extract are catechins, which comprise four major epicatechin derivatives; namely, epicatechin, epigallocatechin, epicatechin gallate, and EGCG [112]. Other components include three kinds of flavonoids, known as kaempferol, quercetin, and myricetin [113].

Catechins, have been shown to have potential health benefits in e.g. diabetes, Parkinson's disease, obesity, Alzheimer's disease, or cancer [114]. Numerous scientific publications suggest that EGCG ((2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl) 3,4,5-trihydroxybenzoate), the major catechin in green tea [115,116], may be the main responsible for the majority of the health benefits attributed to tea consumption [89].

Epidemiological, cell culture, animal, and clinical studies link the antitumoral ability of EGCG to its ability as antioxidant [117], cell proliferation inhibitor [117], tumor promotion blocker [119], apoptosis inducer, and angiogenesis and metastases suppressor [36,44].

NFκB is an oxidative stress-sensitive transcription factor, frequently overactivated in cancer cells, which may be inactivated by EGCG [44]. Apoptosis induction by EGCG through NFκB inactivation has been associated with an enhancement of phosphorylation-dependent degradation of IβB, increases in nuclear translocation of p65 and inhibition of IKK [119]. The induction of negative regulators of the cell cycle may be the consequence of this inhibition. EGCG would then act as an apoptosis inducer in a mechanism involving decreased expression of cyclin D1, cyclin E, CDK2, CDK4, CDK6, and the phosphorylation of retinoblastoma protein [44]. Furthermore, NFκB promotes transcriptional up-regulation of the antiapoptotic proteins Bcl-2 and Bcl-XL. Therefore EGCG, through negative regulation on NFκB, can decrease the levels of anti-death proteins and thereby favor apoptosis induction [120]. Besides, EGCG may enhance the expression of proapoptotic proteins and caspase-3 activity [121] and p53 stabilization [81], thus further favoring apoptosis.

Curcumin

Curcumin, a phenolic compound derived from rhizome of the plant *Curcuma longa*, is a spice originally used in India to add a specific flavor and yellow color to food. In nature curcumin [bis-(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione, commonly called diferuloylmethane] is a bis- α,β unsaturated β -diketone exhibiting a keto-enol tautomerisms that coexists at equilibrium in plants [122]. This substance has been traditionally used, as an anti-inflammatory, in eastern medicine [122,123]. Nowadays, due to its health benefits and the results obtained in clinical trials, curcumin has received the GRAS status (generally recognized as safe) by the U.S. Food and Drug Administration [123].

Curcumin has potential anticancer activity due to its antiradical, immunomodulatory, antiangiogenic properties, antiproliferative, and proapoptotic acting at multiple levels via pleiotropic effects on genes and cell signaling pathways [89, 123]. The antioxidant activity can arise from either the hydroxyl group or the methylene group of the β -diketone moiety [124,125]. *In vitro* and *in vivo* experiments have shown the ability of curcumin to sensitize tumor cells to TRAIL-induced apoptosis, inhibit NFκB activity, and downregulate expression of the antiapoptotic Bcl-2, Bcl-xL, and XIAP proteins [126,127]. Moreover, curcumin upregulates expression of P53, Bax, Bak, PUMA, Bim, NOXA and death receptors DR4 and DR5, triggering activation of caspase-3, -9 [77], -7, and inducing polyadenosine-5' diphosphate-

ribose polymerase (PARP) in e.g. mantle cell lymphoma and multiple myeloma cell lines [128,129].

The Death Associated Protein kinase 1 (DAPK1) is a well-known tumor suppressor gene with pro-apoptotic properties [130,131] that is down-regulated in many human cancers. Indeed promoter hypermethylation reduces expression and function of DAPK1 in numerous cancer cells [132]. Recently, Wu et al. [133] have demonstrated that curcumin modulates gene expression in glioblastoma U251 cells and increases cellular levels of DAPK1 [133]. Although there are contradictory results on the relationship between DAPK1 levels and NF-κB activity [134,135], Wu et al. [133] show that knockdown of DAPK1 expression rescues the inhibitory effect of curcumin on NF-κB phosphorylation. Thus, to avoid the inhibitory effect of curcumin on NF-κB DNA-binding activity, Wu et al. illustrates how DAPK1 knockdown, after curcumin treatment, reduces the activation of caspase-3 in U251 cells [133]. Moreover, Signal Transducer and Activator of Transcription 3 (STAT3), a transcription factor that plays an important function in cell growth and apoptosis regulation and is constitutively activated in glioma cells [136], is inhibited by curcumin [133]. The authors suggest that inactivation of STAT3 is attenuated by DAPK1 knockdown, thus indicating the connection between both factors [133]. So, although further investigations are required to know the exact mechanisms underlying DAPK1 regulation by curcumin, pro-apoptotic effects of curcumin involve DAPK1 overexpression regulating STAT3 and NF-κB pathways and caspase-3 inhibition [133].

Pterostilbene

Pterostilbene (*trans*-3,5-dimethoxy-4-hydroxystilbene) is natural resveratrol analogue but significantly more bioavailable in plasma when ingested [137]. As is the case with resveratrol it is also found in the grape skin, although most abundantly in blueberries [137]. Although their pharmacological properties are very similar, pterostilbene presents several advantages over resveratrol. The two methoxy groups of the pterostilbene molecule increase its lipophilic character and oral absorption, and facilitate its cellular uptake [137]. Actually, the difference in chemical structure is extremely important since stilbene bioactivity and bioavailability are tightly correlated issues.

Pterostilbene has been shown to induce apoptosis in different cell lines both *in vitro* and *in vivo* experiments. It is able to induce mitochondrial membrane depolarization with a subsequent activation of the caspase cascade in cancer cell lines from different origins. Breast (MCF7) [83,95,138], bladder (T24) [139], colon (HT29) [95], myeloid leukemia cells (HL-60, K562) [140], human T lymphoma (HUT78) [140], multidrug resistant myeloid cell lines (HL60-R, K562-ADR) [140], lung cancer cell lines (A549, H460, SK-MES-1) [97,143], melanoma (A375, SK-MEL-2, MeWo) [95,144], pancreatic cancer (MIA PaCa, PANC-1)[143], prostate (PC3) [83], and human gastric carcinoma cells [145]. Furthermore, pterostilbene reduces the levels of antiapoptotic proteins Bcl-2 [84, 85, 141] and Bcl-xL [141] and upregulates the proapoptotic proteins Bax [82-84,144], Bak [83], Bad [83, 144], and Bid [84]. Although these results are consistent with activation of the intrinsic pathway, the apoptotic mechanisms induced by pterostilbene are not completely understood. To determine whether pterostilbene-induced apoptosis is limited to the mitochondria-dependent pathway, a Caspase-9 inhibitor (*z*-LEHD-fmk) and a pancaspase inhibitor (*z*-VAD-fmk) have been used in combination with pterostilbene. The results of combined treatments did not prevent apoptosis induction in leukemia, melanoma, lung, colon or breast cancer cell lines [95,140]. Thus, suggesting that pterostilbene induces

apoptosis through a caspase independent mechanism. In fact, studies in stomach cancer and leukemia cells show that pterostilbene induces the Fas receptor-mediated mechanism of apoptosis (extrinsic pathway), which suggest that a dual mechanism may coexist [140,144]. Other studies demonstrate the ability of pterostilbene to inhibit cell growth by inducing cell cycle arrest [84,95,140] and to alter expression of cell cycle regulators such as P53 and retinoblastoma protein. Nevertheless, anticancer effects of pterostilbene are far of being completely understood. Apoptosis and autophagosome accumulation in cancer cells of various origins are not determinant in cell demise. Pterostilbene promotes cancer cell death via a mechanism involving lysosomal membrane permeabilization, and different grades of susceptibility were observed among different cancer cell lines depending on their lysosomal heat shock protein 70 (HSP70) content (a known stabilizer of lysosomal membranes) [95].

In vivo Administration, Metabolism, and Bioavailability

Although dietary phenolics show diverse pharmacological potentials, their poor oral bioavailability minimizes, and even precludes, real efficacy as therapeutic agents [145]. Most orally administered polyphenols cannot be absorbed from the intestine in their native form, so they are rapidly and extensively conjugated before to gain access to the blood circulation. Coexisting compounds in the lumen, inhibition of digestive enzyme activity and/or alteration of intestinal transport system can modulate their intestinal absorption [47]. Once absorbed, polyphenols are further metabolized in the liver [146] and then eliminated through urine and bile.

Polyphenols are subjected to 3 main types of conjugation: methylation, glucuronidation and sulfation [147]. This extensive conjugation followed by a rapid excretion of the conjugated metabolites is responsible of their poor bioavailability.

In the case of methylations, some polyphenols contain O-methylations in its native form. While some studies suggest that multiple enzymes-mediated methylations can increase the bioavailability of polyphenols, other studies indicate a marked decrease in the anticancer benefits of methylated polyphenols [82,148,149]. On the other hand, glucuronidation and sulfation represents the main clearance pathways for most polyphenols. Glucuronidation is mediated by UDP-glucuronosyltransferases (UGT) [145], but it has been shown that glucuronides of different natural polyphenols are biologically much less active than their native form [145,147]. Sulfation, is catalyzed by sulfotransferases (SULTs) and, as it occurs with glucuronidation, sulfated metabolites also loss biological activities as compared with the natural structures [150].

Taken together all available experimental data indicate that polyphenol aglycones, when absorbed by intestinal enterocytes, undergo extensive phase II metabolism via UGT isoforms which markedly decrease the amount of unconjugated/natural compounds reaching the systemic blood circulation [145]. Moreover, hydrophilic polyphenol conjugates need carriers to cross the enterocyte membrane on the luminal (MRP2) and the serosal side (MRP3 and MRP4) [151]. However, these limitations can be circumvented by intravenous administration. Once in the blood circulation, polyphenol aglycones reach the liver, where they are rapidly metabolized to methylated, glucuronidated, and/or sulfated conjugates.

Due to the extensive conjugation of natural polyphenols, glucuronic and sulfate conjugates have been proposed as potential responsables of some polyphenols-induced effects. In fact, numerous studies have

been conducted with some polyphenols as resveratrol [152-155], quercetin [156-159], curcumin [160,161] or EGCG [162,163] and their metabolites in order to test and compare their effects. Therefore, with only a very few exceptions, most available data indicate that natural polyphenols are biologically more active than their metabolites.

In addition to their rapid metabolism, under *in vivo* conditions, the chemical stability and solubility of polyphenols are critical, as they have limited water solubility. These factors further complicate the use of natural polyphenols in a clinical setting [164]. Currently, there are some challenging works aimed to improve bioavailability using delivery systems such as liposomal formulations [165], nanoparticles, microemulsions and polymeric implantable devices which have demonstrated to deliver therapeutic concentrations of various polyphenols into the systemic circulation [166].

Conclusions

The identification of key molecular components in the apoptotic pathway has provided rationale targets for the development of new anticancer therapeutics. However, synthetic molecules are not the only alternative. Evidences from experimental *in vitro* and *in vivo* studies, and a few clinical trials, suggest polyphenols as effective anti-cancer agents, both in the form of nutritional supplements or functional foods, and as potential anticancer drugs. Some mechanisms by which polyphenols have been shown to prevent carcinogenesis include inhibition of pro-inflammatory molecules generation, oxidative stress, DNA damage, and cancer cell proliferation, and by increased apoptosis. In addition, polyphenols show synergic/additive effects if administered (as a complementary adjuvant therapy) in combination with conventional anticancer chemotherapy and/or radiotherapy. Polyphenols such as resveratrol, EGCG, curcumin and pterostilbene, have been shown to promote extrinsic and intrinsic apoptosis induction in different types of cancers (e.g. colon, lung, prostate, breast, melanoma or leukemia). Upregulation of proapoptotic proteins, downregulation of antiapoptotic proteins, NFκB induction, and p53 activation, are key mechanisms underlying the ability of polyphenols to induce apoptosis in cancer cells. However, more studies must be done to fully understand how polyphenols may be used to induce cancer cell apoptosis under *in vivo* conditions. Parallely, improvements in delivery systems to increase their bioavailability, stability, and solubility, are necessary in order to make possible the use in clinical settings.

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