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Journal of Nutritional Biochemistry 16 (2005) 449–466

Journal of Nutritional Biochemistry

Resveratrol as an anticancer nutrient: molecular basis, open questions and promises

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Received 17 September 2004; received in revised form 11 January 2005; accepted 20 January 2005

Abstract

The polyphenol resveratrol is an anticancer nutrient that was shown to inhibit cancer initiation and promotion [Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997;275:218-20]. The absorption, transport and metabolism of resveratrol will be reviewed as well as its actions in multiple pathways involved in the regulation of the cell cycle and the induction of apoptosis. Resveratrol acts as a selective estrogen receptor modulator (SERM) and regulates proteins involved in DNA synthesis and cell cycle, such as p53 and Rb/E2F, cyclins, cyclin-dependent kinases (CDKs) and their inhibitors. Resveratrol affects the activity of transcriptional factors involved in proliferation and stress responses, such as NF-kB, AP1 and Egr1. Part of these events is mediated by mitogen-activated protein kinases (MAPKs) and tyrosine kinases (e.g., Src) and leads to the modulation of survival and apoptotic factors [e.g., Bcl2 family members, inhibitors of apoptosis (IAPs), ceramide] as well as enzymes involved in carcinogenesis [cyclooxygenases (COXs), nitric oxide synthase (NOS), phase I and II enzymes]. Moreover, resveratrol affects the expression and the activity of cotranscriptional factors such as p300 and sirtuin 1. Thus, resveratrol potential as an anticancer chemopreventive and chemotherapeutic agent and its implication in the prosurvival versus prodeath pathway induction will be discussed.

Keywords: Resveratrol; Cancer; Nutrient

1. Introduction

Food intake modulates metabolism and health, and supplies a variety of bioactive molecules necessary for life. Throughout the ages, medicine has fought against dietary deficiencies. In the 1800s, vitamin deficiencies caused diseases such as scurvy, whereas today, a low intake of phyto-antioxidants, in association with an excess intake of saturated fats in the Western diet, is linked to cardiovascular disease and cancer. During the past 50 years, advances in medical research have led to the development of an extraordinary array of synthetic molecules designed to offer specific cures. However, diseases such as cancer are not easily treated with a single antitumor agent, since they develop from multistep processes and vary according to the genetic and epigenetic characteristics of individuals. Knowledge of the beneficial health effects of natural compounds present in vegetables, herbs and roots is an instinct for animals and an ancient tradition for humans. Western countries abandoned such traditions in favor of modern food and drug production, but Chinese and Japanese traditional medicines developed this knowledge over the centuries, and accumulated experience and epidemiological observations that represent today our best insight into cancer therapy.

Natural compounds are administered orally and are normally absorbed and metabolized. It is still unknown whether natural compounds are most effective when assumed daily for cancer prevention or when taken at higher doses and in specific combinations aimed at the cure. It is believed that a varied diet is advantageous in that it offers a mix of natural compounds, achieving additive or even synergistic effects.

Natural compounds fit into mechanism-based approach that targets whole pathways and sets of intracellular events rather than a single enzyme, as do many synthetic drugs. This offers a less specific but perhaps more effective strategy for cancer therapy by inducing combinations of

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doi:10.1016/j.jnutbio.2005.01.017

effects that may counteract the metabolic alterations related to cancer promotion.

This paper explores the latest evidence for the antitumor properties of resveratrol, a polyphenol produced by plants and most known for being responsible for the beneficial health effects of wine.

2. The polyphenol resveratrol

Polyphenols comprise a large class of antioxidants and include flavonoids, anthocyanins, phenolic acids, lignans and stilbenes. These compounds are all derived from phenylalanine and contain an aromatic ring with a reactive hydroxyl group. Within the subclass of stilbenes, resveratrol is the common term for 3,5,4′ -hydroxystilbene. Resveratrol exists in both trans and cis isomeric forms (Fig. 1). Since the trans isomer is by far more commonly found in plants and extensively studied, throughout this review, the term resveratrol refers to 3,5,4′ -*trans*-hydroxystilbene.

In plants, polyphenols (including resveratrol) are generally found as 3-*O*-β-D-glucosides, called piceids (Fig. 1). Other minor conjugated forms of resveratrol contain 1-2 methyl groups (e.g., 3,5-trimethoxy-4′ -hydroxystilbene, called pterostilbene), a sulfate group or a fatty acid. Interestingly, substitutions involve a maximum of two hydroxyl groups, and thus conjugated polyphenols maintain their antioxidant properties. A naturally occurring analog of resveratrol that carries four, rather than three hydroxyl groups, is piceatannol (3,4,3′ ,5′ -*trans*-tetrahydroxystilbene).

2.1. Biosynthesis in plants

Resveratrol is produced by a restricted number of plants (about 31 genera). It is not normally present in large amounts, and it is produced in response to stress; in fact, resveratrol belongs to a class of defense molecules called phytoalexins that protect against infection and damage from exposure to ultraviolet (UV) irradiation [1,2]. Resveratrol is toxic to plant pathogens, but some parasites such as fungi overcome this toxicity through the action of membrane proteins (ABC transporters) that transport the compound out of the cellular compartment [3]. Overproduction of stress response molecules in plants triggers a hypersensitivity reaction that can lead to cell death when the stress cannot be counteracted [4].

Resveratrol biosynthesis, catalyzed by stilbene synthase, consists in the repetitive decarboxylative condensation of a *p*-coumaroyl residue from *p*-coumaroyl-CoA with three C_2-units from malonyl-CoA (Fig. 2). Further reactions conjugate native resveratrol to glucosyl or sulfate residues at the 3-position of the biphenolic ring.

Resveratrol is susceptible to oxidative degradation, while the glycosylated piceid form is resistant. Glycosylated resveratrol maintains its biological activity, is more stable and soluble, and therefore is more readily absorbed by the human intestine [5].

2.2. Natural sources

Resveratrol and the analogs piceatannol and pterostilbene are found in several edible natural products such as grapes (*Vitis* spp.), peanuts (*Arachis* spp.) [6], berries (blueberries, cranberries and lingonberries, all *Vaccinium* spp.) [7] and rhubarb (*Rheum* spp.) [8]. Resveratrol was first detected in the dried roots of *Polygonum cuspidatum* (Itadori tea), traditionally used in Chinese and Japanese medicines as an antiinflammatory agent [1,9]. Moreover, its occurrence in the plant kingdom is widespread, being present in wild nonedible berries of *Vaccinium* spp. [7] and in *Eucalyptus* spp. [10], *Yucca schidigera* [11], *Dracaena loureiri* [12], *Cassia* spp. [13], mulberry (*Morus* spp., *Maclura pomifera, Nothofagus fusca* spp.), and red sandalwood (*Pterocarpus* spp., a major source of pterostilbene) [7]. Extracts from roots, heartwood, bark and leaves of most of these plants are commonly used in traditional oriental medicine.

The content of resveratrol in different sources varies widely, depending on factors such as cultivar, climate, fungi infections, UV exposure and wine-making procedures.

2.3. Biological activities of resveratrol: from plants to humans

Many classes of phytochemicals exert antioxidant activities as well as other beneficial effects, for example, on the inflammatory responses, on cellular enzymatic detoxification systems and on proliferative and apoptotic factors. The human diet contains a mixture of plant-derived polyphenols such as genistein, quercetin, epigallocatechin (from soybeans and green tea leaves) and resveratrol, mainly present in grape skin and peanuts. The biological activities of these substances have been demonstrated in humans [14,15]. In the early 1970s, epidemiological studies revealed an inverse correlation between red wine consumption and cardiovascular diseases in France [16]. The "French paradox" was resolved with the identification of antioxidants such as resveratrol in wine. Subsequently, a large number of studies identified multiple beneficial effects of this molecule in humans [16]. In plasma, resveratrol was associated with lipoproteins [17] and it was shown in vitro to inhibit low-density lipoprotein (LDL) oxidation [18,19]. In vitro studies documented that resveratrol inhibited platelet aggregation [20] and polymorphonuclear cell activation (production of reactive oxygen species) [21]. In human endothelial cells, resveratrol induced vasorelaxation [22] and impaired migration and tube formation, thus reducing thrombogenic potential, by inhibiting expression of adhesion molecules [21,23,24].

In the last decades, phenolic acids (e.g., gallic acids, caffeic acids, tannic acids, curcumin), flavonoids (genistein, daidzein, quercetin, myricetin, kaempferol) and other polyphenols (epigallocatechins) were shown to induce apoptosis in cancer cells [14], and a few clinical trials using these natural products have been carried out [25,26]. In 1997, Jang et al. [13] reported that resveratrol exerts antitumor

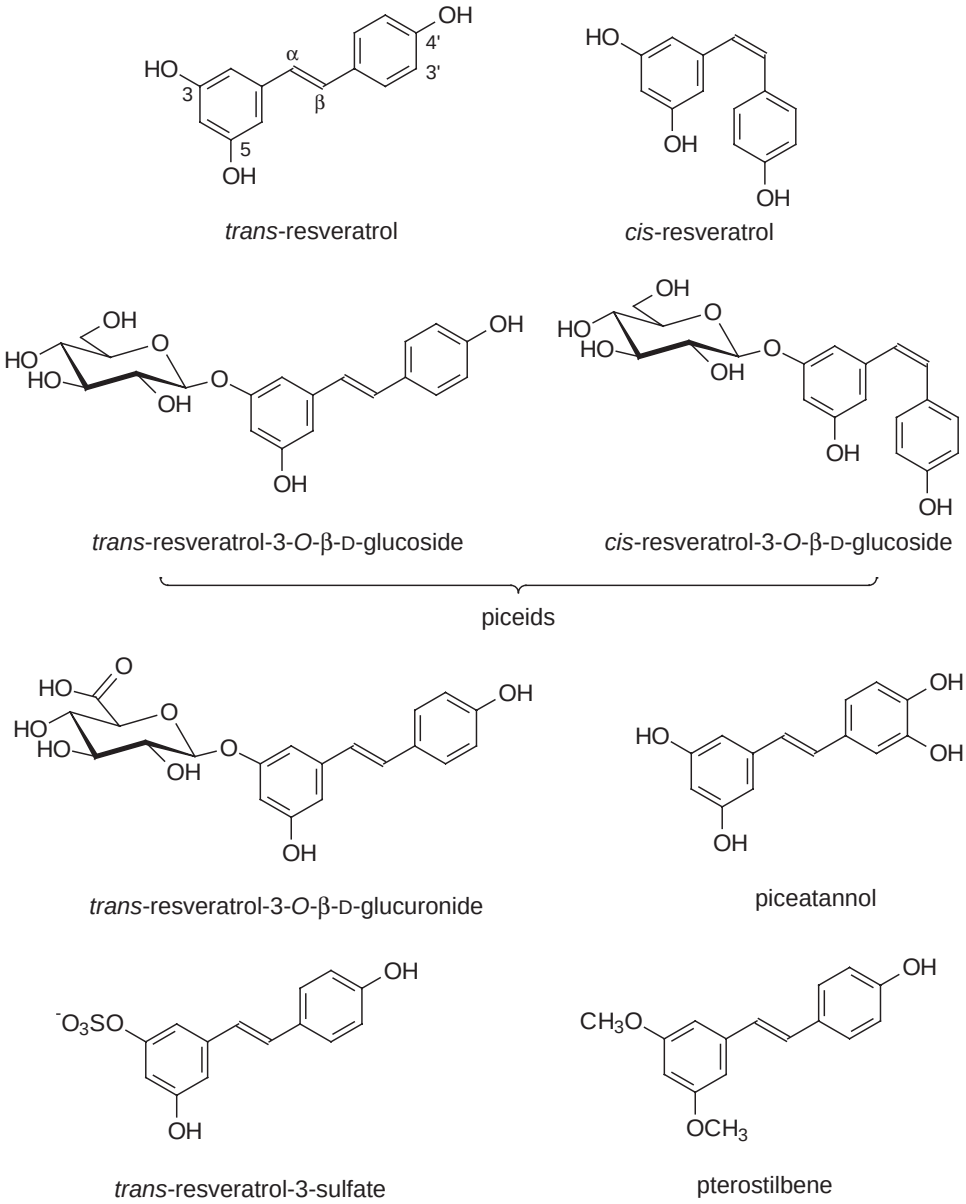

Fig. 1. Structural formulas of resveratrol and its most common conjugates and analogs.

properties by reducing tumor mass in rats. These authors demonstrated that resveratrol is effective in blocking in vivo the three stages of carcinogenesis: initiation, promotion and progression. Since this pioneer work was published, many studies employing human cancer cell lines have confirmed this observation and have sustained that resveratrol is a chemopreventive agent [27]. Less convincing and still to be established is its chemotherapeutic potential.

3. Resveratrol in the diet: absorption, transport, metabolism and excretion

In order to understand the potential of specific and beneficial properties of dietary compounds, it is useful to study their absorption after oral intake, transport in body fluids, cellular metabolism and, ultimately, excretion. The absorption and transport of resveratrol have been studied in several models: isolated rat intestine [28,29], rats and mice after oral administration [30–34], human colon carcinoma Caco-2 cell line [35], human hepatocytes [36] and healthy human subjects [31,37]. The complex pathways for resveratrol absorption, transport, metabolism and excretion are summarized in Fig. 3.

3.1. Absorption

Experiments with isolated rat intestine perfused in vitro with resveratrol-containing buffer showed that jejunum and, to a lesser extent, ileum are involved in the absorption of resveratrol [29]. Most of the resveratrol transported to the basolateral side was in a metabolized form mainly as

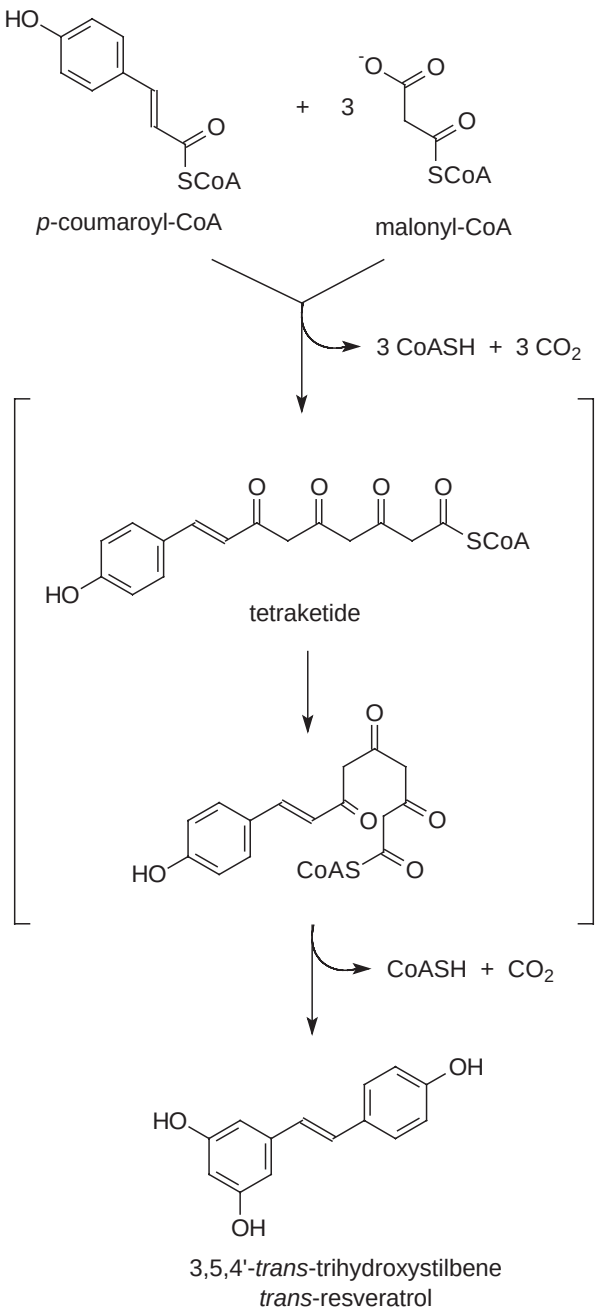

Fig. 2. Biosynthetic pathway of resveratrol.

glucuronide and also as sulfate conjugates. However, the total amount of resveratrol and its conjugates crossing the intestinal epithelium was only 6% of the total perfused amount [29]. The results of this in vitro study contrast with the higher intake observed in vivo, in both animals and humans; for example, in mice fed orally with resveratrol, up to 75% of the ingested content was absorbed and the remainder was eliminated in the feces [31].

In vitro studies showed that resveratrol is metabolized also by human intestinal cells. The human colonic adenocarcinoma cell line Caco-2, treated in vitro with resveratrol, exhibited initially a dose-dependent increasing rate of apical to basolateral transport, until this reached a plateau with a maximal transport that is not concentration dependent [35]. Reverse transport may also occur when cells are incubated with resveratrol on the basolateral side. Resveratrol is released from enterocytes in a conjugated soluble form. In this study on human cells, resveratrol was released mainly as sulfatide conjugate and only in minimal amount as glucuronide conjugate [35], whereas in rats, the majority of the resveratrol metabolism involves glucuronidation [29,31].

Resveratrol is present in dietary products as *cis*- and *trans*-resveratrol but mainly in glycosylated forms named piceids (3-*O*-β-D-glucosides). Plants and pathogens as well as the human digestive tract contain enzymes that oxidize polyphenols. Glycosylation inhibits enzymatic oxidation of resveratrol, thereby preserving its biological activity and increasing its stability and bioavailability [5,38]. Since intestinal cells are only able to absorb aglycone resveratrol, the absorption process requires glycosidases [39]. Therefore, the relative amounts of aglycone and glycosylated resveratrol in foods may modulate the absorption rate. Different activities and expression levels of intestinal glycosidases may explain the differences in resveratrol uptake rates observed in humans and rats.

3.2. Transport to tissues

Conversion of resveratrol into hydrophilic conjugates may facilitate its entry in the blood stream, its diffusion throughout the body and, most importantly, its excretion.

In vivo studies in animals showed that after the oral administration of resveratrol, both aglycone and conjugated forms appeared in plasma. With time, plasma concentration diminished until a secondary peak appeared. This secondary peak was due to the recirculation of resveratrol after release from bile. The liver and gallbladder filtered resveratrol and its metabolites from the circulation and transported them back again into the intestine through the bile for a delayed absorption [30,33]. Shortly after oral intake, resveratrol was found in colon, whereas its tissue distribution required a few hours. In liver, resveratrol accumulated up to a concentration comparable to that which exerts biological effects in in vitro assays (micromolar range) [34].

The uptake and metabolism of resveratrol by human liver have been studied in in vitro models. Human hepatocytes exhibited an initially increasing rate of uptake (minutes), then the rate remained stable (hours) [36]. At low concentrations, hepatocyte uptake was mediated by temperature-sensitive active transporters (half saturation at 30 μM). At higher concentrations, the molecule diffused into cells in a nonsaturable but concentration-dependent process [36].

3.3. Metabolism and excretion

In animal model, renal excretion of resveratrol started within hours after intake and increased throughout the next 12–24 h [34]. The presence in urine of little native (aglycone) resveratrol but high amounts of its conjugates

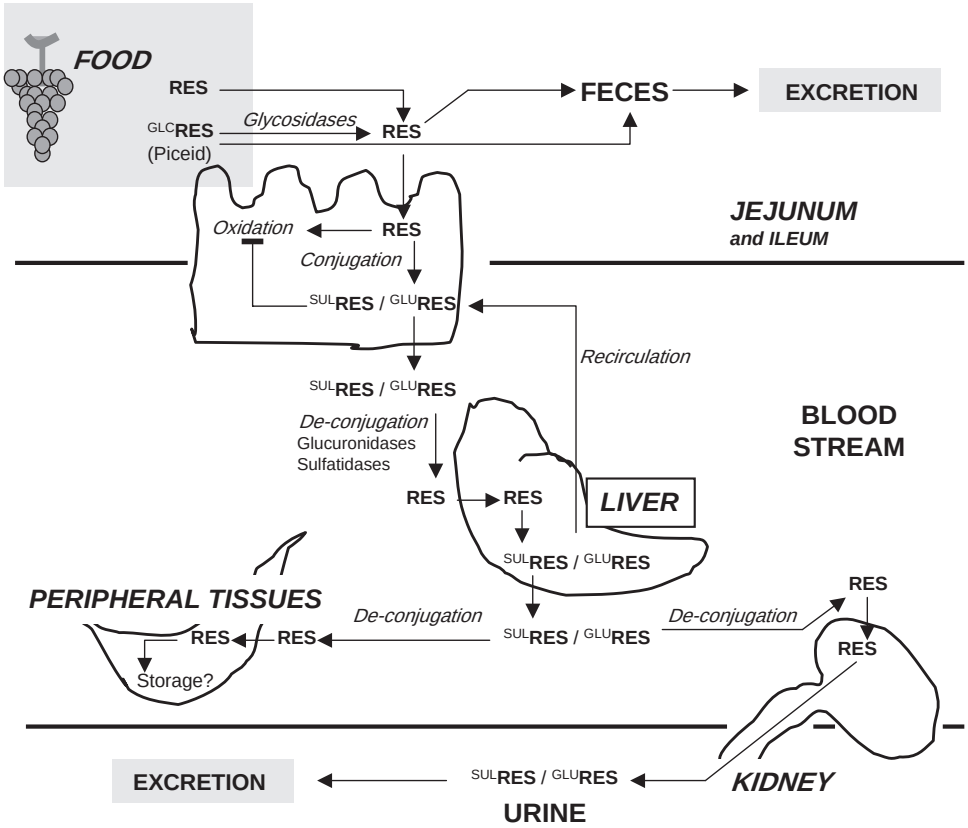

Fig. 3. Pathways of resveratrol absorption, transport, metabolism and excretion. ^{GLC}RES, resveratrol-3-*O*-β-glucoside (piceid); ^{SUL}RES, resveratrol-3-sulfate; ^{GLU}RES, resveratrol-3-*O*-β-glucuronide.

indicates that metabolism of the compound is essential for excretion [33]. In kidney, resveratrol was present mainly in its native form, whereas in urine, the majority of the compound was present in its conjugated form [34].

In human, the excretion time depended on the concentration of resveratrol (aglycone and conjugates) present in plasma, but there was no direct correlation between excreted and introduced amount. This observation from Meng et al. [40] suggests that while small amounts of resveratrol are rapidly metabolized and eliminated, retention and accumulation of the compound in tissues occur only over a certain dose of intake, thus becoming potentially available for cellular uptake and intracellular signaling.

Conjugation with glycidic or sulfatidic groups is probably aimed at promoting excretion, since conjugates are not intracellularly active [41]. Flavonoids such as quercetin inhibit the glucuronidation of resveratrol and therefore may increase its bioavailability [42]. This observation may partly explain why low concentrations of dietary compounds have synergistic effects [43].

Many questions regarding resveratrol metabolism and excretion remain unanswered. It is still to be clarified if resveratrol conjugates are transported to tissues or if they are only targeted for excretion. Moreover, since liver uptake has only been studied in vitro using the native aglycone form of resveratrol, it is not known whether hepatocytes also absorb the conjugated form of resveratrol from the blood stream or if these conjugates are targeted for excretion.

4. Antitumoral activity of resveratrol

Antitumor agents impair procarcinogen metabolic activation and interaction with cellular targets (DNA, proteins); they inhibit cancer development by blocking tumor cell transformation and proliferation and by inducing tumor cell death [14]. Therefore, chemoprevention and chemotherapy can be obtained by acting at multiple levels and by impairing combinatorial effects responsible for mutagenesis and mitogenesis. Among food-derived molecules that have been screened for the ability to inhibit or reverse such cellular processes, resveratrol is particularly interesting because it affects a broad range of intracellular mediators involved in the initiation, promotion and progression of cancer [13,15,27]. Since Jang et al. showed in vivo the antitumor potential of resveratrol, many studies revealed a variety of resveratrol intracellular targets whose modulation give rise to overlapping responses that lead to growth arrest and death. Anyway, the efficacy of this molecule is still debated because of the multiplicity of affected targets and contradictory effects related to dose and time of treatment

and to cellular phenotype. The specific cell molecular setting determines the response to resveratrol treatment. In this review, the action of resveratrol on tumor genesis and growth will be examined and discussed.

4.1. Resveratrol: a phytoestrogen with agonistic and antagonistic hormonal activities

Loss of estrogen and androgen production in aging leads to deregulated functioning of tissues and organs. Moreover, improperly balanced hormonal stimulation may favor cell proliferation over differentiation and senescence, and may increase the risk of developing cancer. Consequently, hormone-dependent tumors (breast and prostate, but also others such as colon and lung) may be prevented by the daily intake of appropriate amounts of selective estrogen receptor modulators (SERMs). These compounds exhibit various degrees of estrogen agonism or antagonism, depending on the cell type, expression of genes targeted by estrogen receptors (ERs) and other related intracellular responses [44].

Several polyphenols structurally resemble estrogens. Phytoestrogens such as the flavonoid quercetin and the isoflavone genistein behave as both agonists and antagonists of estrogens receptors, giving rise to opposing responses according to concentration, competition and ER expression [45]. Breast cancer incidence among women in Western countries is sixfold higher than that among women in Asia who consume daily soy products rich in phytoestrogen, suggesting that these latter may act as chemopreventing agents [46].

The polyphenol resveratrol can be considered as a dietary phytoestrogen with powerful beneficial effects on both ER-expressing and nonexpressing human tumors. The chemical structure of resveratrol is similar to that of the synthetic estrogen diethylstilbestrol (4,4′ -dihydroxy-*trans*-diethylstilbene). Resveratrol belongs to the type I class of estrogens [47]. It binds ERs in the low micromolar range with an affinity lower than that of estradiol; therefore, it behaves as a weak competitor. Despite the lower binding affinity, resveratrol may act as a superagonist in activating hormone receptor-mediated gene transcription [47,48]. The superagonistic induction of gene expression is related to the promoter context and varies according to cell type [49].

Aside from superagonism, resveratrol also exerts an antiestrogen action by triggering parallel pathways that inhibit estrogen-induced cellular outcomes, such as proliferation, tumoral transformation and progression [13,50,51]. In the presence of estrogen, resveratrol exerted a mixed agonistic–antagonistic action in ER-positive breast cancer cells [52]. Resveratrol binding to ERβ suppressed the expression of the α form of ER [53] and regulated androgen receptor signaling by repressing receptor coactivators and downstream gene transcription [54,55]. Kuwajerwala et al. [56] reported that in androgen-sensitive prostate cancer cells, resveratrol induced a proliferative effect at low dose (5 μM) but an apoptotic effect at 15 μM or higher dose, whereas in androgen-independent cells, resveratrol did not increase DNA synthesis at any concentration. It has been proposed that resveratrol inhibition of focal adhesion kinase (FAK) and protein kinase B (PKB/Akt) is responsible for inducing apoptosis in ER-positive breast cancer cells [57]. In this study, apoptosis occurred only in ER-positive cells but it did not involve receptor-mediated gene transcription. Steroids (both estrogens and androgens) and their receptor complexes modulate mitogen-activated protein kinases (MAPKs) [by activating ERK, down-regulating Jun N-terminal kinases (JNK)] and thus downstream transcriptional events [58]. Mitogen-activated protein kinases have been shown to mediate also upstream events induced by resveratrol, leading to downstream regulation of transcriptional factors [59–61]. In breast cancer cells, 17β-estradiol reversed resveratrol-induced apoptosis even if estrogens and resveratrol acted similarly in stimulating MAPKs (specifically ERKs), either when administered alone or in combination (additive effect) [62].

This contrasting evidence may be explained by considering resveratrol ability to alter nongenotropic activities of steroid receptor complexes. Activated ER may rapidly stimulate the activity of G proteins and protein kinases, independently of gene transcriptional events [63].

Resveratrol may therefore be considered as a natural SERM [49], although the balance between prosurvival genotropic and opposing nongenotropic activities is not clearly predictable due to the role of a broad array of intervening factors. At the low doses provided by dietary intake, resveratrol may act as a weak estrogen competitor, according to the receptor expression and hormonal status of tissues; it counteracts the proliferative effects of hormones and provides a balancing antitumoral activity. Tissue-specific expression of α and β ERs, cofactors regulating DNA binding and different gene promoters, all modulate the cellular response to resveratrol. In the absence of endogenous hormones and according to cellular specificity, the superagonistic activity of resveratrol may act in an opposite manner and prevent tissue senescence and apoptosis. When stress signals overcome proliferative signals, or when these latter are missing (absence of hormones), the polyphenol-induced pathway may switch to apoptosis (Fig. 4).

Aggressive breast and prostate tumors often lose ER expression and become estrogen independent. However, resveratrol is also able to affect ER negative cells. Besides binding hormone receptors, resveratrol is able to trigger intracellular events that affect different metabolic pathways. Indeed, resveratrol exhibits antitumoral properties in a variety of cells that do not express steroid hormone receptors [51,52,64], as well as in cells treated with ER blockers (e.g., tamoxifen) [65]. These observations indicate that resveratrol triggers multiple pathways that may or may not involve ER activation. In this sense, resveratrol may be considered as both a chemopreventive and a chemotherapeutic agent.

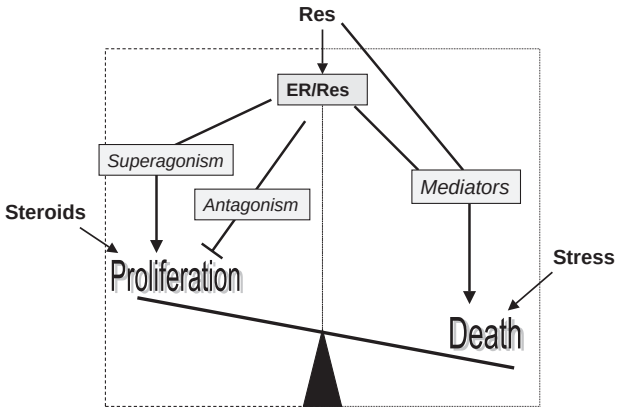

Fig. 4. Pathways in which resveratrol acts as a SERM.

4.2. Resveratrol: a modulator of phase I and phase II enzymes

Cancer initiation occurs as a consequence of multiple events. The combined effects of stimulating factors (e.g., hormones, cytokines), stress mediators (inflammatory oxygen radicals) and exogenous aggressions (viruses, radiation and xenobiotic compounds) can affect the control of cellular proliferation and lead to the tumoral transformation of tissues.

Xenobiotics (carcinogens and drugs) are often lipophilic substances that easily enter cells. In order to protect from their toxicity, cells render them more hydrophilic and excretable through a process called biotransformation. This process involves oxidation by phase I enzymes and conjugation with polar groups by phase II enzymes. However, this process may lead to the formation of highly reactive oxygen radicals and other molecules that easily interact with DNA and thus procarcinogens become converted into carcinogens.

The oxidative phase involves membrane-bound enzymes (e.g., cytochrome P450 monooxygenase, CYP) that use molecular oxygen to introduce an oxygen atom into substrates [66,67]. The second phase is carried out by transferases that add hydrophilic groups or change the redox state of the molecule (e.g., glutathione-*S*-transferase, UDP-glucuronosyltransferase, sulfotransferase, NAD(P)H:quinone oxidoreductase). Chemopreventive strategies include the inhibition of phase I enzymes responsible for activating xenobiotics and the induction of phase II enzymes that conjugate these activated compounds to endogenous ligands (e.g., glutathione).

Resveratrol, being an exogenous lipophilic compound, can cross plasma membrane, be subjected to cellular metabolism and it possibly interacts with phase I enzymes. Resveratrol inhibited human recombinant CYP P450 in vitro [41]. Moreover, it inhibited CYP450 activity from mouse or human liver microsomes [68,69]. Jang et al. [13] found that resveratrol reduced the insurgence of preneoplastic lesions in mouse mammary gland cultures and decreased the incidence of tumor formation in mice treated with 7,12-dimethylbenz[*a*]anthracene (DMBA) used as tumor initiator, in combination with phorbol esters used as tumor promoter. Since DMBA requires bioactivation by phase I enzymes CYP1A1, CYP1A2 and CYP1B1 [70], the antitumoral activity of resveratrol in vivo includes prevention of the initiation phase of carcinogenesis by inhibiting phase I enzymes. Resveratrol was found to be able to discriminate among CYP isoenzymes: it inhibited CYP1A1 and CYP1B1 activities directly while it inhibited CYP1A2 indirectly. In this study, it is suggested that indirect CYP inhibition is due to a compound derived from resveratrol metabolism in the presence of NADPH [71].

Besides inhibiting CYP activity, resveratrol also acted at the transcriptional level by blocking the activation of CYP1A1 promoter and gene transcription in human hepatoma cells [69]. In addition, resveratrol impaired the carcinogenic effect of aryl-hydrocarbons. Aryl-hydrocarbons are carcinogens that act via nuclear receptors to promote CYP transcription for the enzymatic conversion of xenobiotics in carcinogen elements. Resveratrol, without binding the receptors, impaired their interaction with the promoter region of the CYP1A1 gene [69].

Resveratrol was further shown to induce phase II enzymes such as UDP-glucuronyltransferase and NAD(P)H:quinone oxidoreductase in mouse epidermis [72].

These data strengthen the hypothesis that resveratrol may be used in cancer prevention. The effects of resveratrol on phase I and II enzymes are summarized in Fig. 5.

4.3. Resveratrol: modulator of nitric oxide synthase

Nitric oxide synthase (NOS) is a heme-containing monooxygenase with a reductase domain and an oxygenase domain. Constitutive isoforms (endothelial, neuronal and mitochondrial NOS) provide low intracellular concentrations of the short-lived free radical nitric oxide (NO). An inducible form of NOS (iNOS), activated at the transcriptional level during inflammation, provides high concentrations of NO

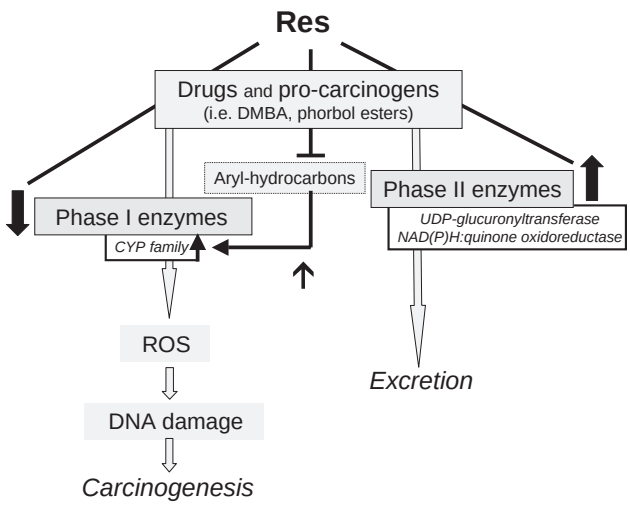

Fig. 5. Pathways in which resveratrol modulates phase I and phase II enzymes.

[73]. NO is known for its vasodilatatory properties [74], its inhibition of platelet adhesion and aggregation [75], and of adhesion molecule expression [76], and its suppression of cell growth and migration [77]. It is thus apparent that NO and resveratrol share common targets.

High levels of endogenously produced NO may either induce apoptosis (by generating toxic reactive intermediates) [78,79] or inhibit it [80,81], depending on the intracellular redox state. The promoter region of iNOS is controlled by transcription factors such as NF-kB, AP1 (Jun/Fos), CREB and STATs. The expression of iNOS is often associated with the induction of tumoral markers such as cyclooxygenase 2 (COX2), vascular endothelial adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1) [82]. Finally, iNOS is up-regulated in cancer cells in relation to tumor progression [73,83].

Resveratrol may favor antitumoral activity by impairing the process of angiogenesis. The compound was shown to inhibit endothelial cell migration and tube formation and to block oxygen radical formation and the related Src-mediated expression of vascular endothelial cadherins [23]. Resveratrol treatment in the micromolar range induced vascular smooth muscle cell relaxation by promoting NO release, and this was reversed by NOS inhibitors. The action was indirect since resveratrol had no effect on NOS activity in rat aortic homogenates measured in vitro. Moreover, metabolic inactivation of NO by NADH/NADPH oxidase was inhibited by resveratrol, thus potentiating the effect [84]. Moreover, resveratrol induced the expression of the endothelial isoform of NOS (ecNOS), in association with the activation of p53 and p21 and cell cycle arrest in S/G2 phase [85].

Conversely, resveratrol was shown to have opposite effects in cancer cells. It inhibited NO production and iNOS expression while it also induced apoptosis in human B-cell lines derived from patients with chronic B-cell malignancies or lymphocytic leukemia [83]. Similarly, in lipopolysaccharide (LPS)-stimulated macrophages, resveratrol reduced iNOS mRNA transcription and cytosolic protein levels, possibly by blocking phosphorylation of I-kB and activation of NF-kB [86].

Although there is conflicting evidence regarding the effect of resveratrol on NO formation, it appears to have antitumoral action via the inhibition of angiogenesis or the induction of cell cycle arrest and apoptosis in cancer cells.

4.4. Resveratrol: a modulator of COX

Resveratrol was first discovered in roots for its ability to inhibit the activity of COXs, enzymes that catalyze the first committed steps of prostaglandin (PG) biosynthesis. Prostaglandins are known stimulators of cell proliferation and angiogenesis, and suppressors of immune surveillance [87]. Cyclooxygenases also possess a hydroperoxidase activity that converts PGG2 to PGH2; this activity, which generates tyrosyl radicals, may be involved in the bioactivation of promutagens, as are phase I enzymes [88].

Resveratrol noncompetitively inhibited both the COX and hydroperoxidase activities of COX1 in vitro [13,89]. This dual effect of resveratrol is unique, since classic nonsteroidal antiinflammatory drugs (NSAIDs) only affect the COX activity [90,91].

Besides constitutive COX1, which is ubiquitously expressed and produces PGs involved in tissue homeostasis, an inducible form of COX2 is expressed during inflammation and neoplasia; COX2 is induced by proinflammatory and mitogenic stimuli [92]. Since overexpression of COX2 inhibits apoptosis and promotes metastasis of tumor cells [93,94], targeting COX2 expression could be a promising chemotherapy strategy.

Resveratrol inhibited human recombinant COX2 in in vitro assays [95]. Resveratrol discriminated between the two COX isoforms, being poor inhibitor of COX2 hydroperoxidase activity [13,89]. The two COX isoforms contribute to the formation of thromboxane A2 (TXA2), and PGI2 from PGH2 [96] and resveratrol, with its selective action, may produce different effects according to tissue expression and functions of the isoforms.

Despite in vitro data on enzyme inhibition, the most relevant mechanism occurring in vivo may likely be a mediated one. Down-regulating COX2 transcription [97], resveratrol was proposed in breast cancer cells to act by inhibiting the activation of the transcriptional factor NF-kB, upstream of COX2 expression [98]. In addition, in a similar cell model, micromolar concentrations of resveratrol blocked phorbol-ester-mediated translocation of protein kinase C (PKC) to the membrane and activation of the COX2 promoter [95]. This study showed that resveratrol inhibited the activation of a cyclic-AMP-responsive element (CRE) that controls COX2 expression, and that mutation of this region abrogated the effects of resveratrol.

The currently available evidence indicates that resveratrol behaves as a tumor-promotion antagonist, by triggering intracellular pathways that oppose the unbalanced expression of COX2.

4.5. Resveratrol-inducted growth arrest and apoptosis in cancer cells

Cancer cells escape from cell cycle control and from G0/G1 terminal differentiation. Resveratrol was found in a variety of cell models to arrest proliferation, mostly in an irreversible way, leading to apoptosis. This effect has been traced to resveratrol ability to modulate the activity of many key mediators of cell cycle and survival.

4.5.1. Cell cycle-regulating proteins

Several protein kinases [cyclin-dependent kinases (CDKs)], their activators (cyclins) and their associated inhibitors form a network of complexes driving cells in and out of the cell cycle phases; their action during the cell cycle is switched off by sequential degradation [99].

A number of studies reported that a variety of different human cancer cell lines, treated with resveratrol at micro-

molar concentrations for 12–24 h, arrested their proliferative cycle in the G1/S boundary [100], in the S phase [98,101–109] or, less frequently, in the G2/M phase [110,111]. One subject of debate is whether resveratrol-induced cell cycle arrest is reversible or is the first step of an irreversible apoptotic program. A number of studies found that the resveratrol-induced cell cycle block did not cause apoptosis [101,103,106,112], was reversible [101,102,112,113] and was associated with cell differentiation in a variety of cancer cells [101,112,114,115].

Cell sensitivity to apoptotic agents may change throughout cell cycle phases [116]; therefore, resveratrol ability to arrest cancer cells in S phase, which is the most vulnerable, may strongly increase its chemotherapeutic potential [109].

Moreover, many authors reported that the induction of cell cycle arrest by resveratrol was followed by apoptotic cell death [56,84,100,104,106–108,111,117,118].

A number of protein targets of resveratrol have been identified. Among these, pivotal roles in cell cycle progression, either in normal or in stressed conditions, are exerted by p53 and by the retinoblastoma gene product (Rb). The p53 oncosuppressor is a DNA-binding protein that activates transcription of genes that induce cell cycle arrest. p53 is present at low levels in normally proliferating cells, and it accumulates, as a result of increased stability due to acetylation and phosphorylation, in response to stress and in senescence [119–122]. p53 arrests the cell cycle in G1 by activating the cyclin inhibitor p21 [123,124]. The outcome of p53-arrested cells is apoptosis mediated by mitochondrial Bcl family proteins, resulting from the increased expression of proapoptotic factors and the activation of caspases [125].

In a variety of cellular models, resveratrol strongly up-regulated p53 and p21, imposing a checkpoint at G1/S transition [84,100,103,106], although the compound was also reported to act independently from p53 cellular status [109]. This altered equilibrium led to the modulation of CDKs and cyclins, both at transcriptional and posttranscriptional levels, resulting in cell cycle arrest in a specific phase [55,56,84,100,102,106,126]. Narayanan et al. demonstrated that resveratrol activated transcription of a whole set of p53-responsive genes (e.g., p21, p300/CBP, Apaf1 and BAK) related to cell cycle arrest and apoptosis, while it down-regulated tumor-associated antigens (e.g., PSA), NF-kB/p65 and Bcl2 [127]. Resveratrol not only increased p53 cellular content but also induced its posttranslational modification (phosphorylation and acetylation) required for regulating gene transcription (such as p21 induction) [62]. Interesting observations have been reported on endothelial cell proliferation and angiogenesis, a process that plays an important role in tumor growth. In vascular endothelial cells, resveratrol activated p53 via serine phosphorylation but without increasing total concentration of p53 or p21 [102]; thus, resveratrol induced a block in DNA synthesis that was not followed by apoptosis. These authors hypothesized that this partial activation of p53 reversibly blocked proliferation because washout of resveratrol restored normal cell cycle progression. In a similar endothelial cell model, other authors observed that resveratrol activated p53 and p21 and induced cell cycle arrest, but this arrest led to apoptosis only in proliferating serum-stimulated cells and not in quiescent cells [103]. This observation is in agreement with other studies reporting that resveratrol was differently toxic to tongue squamous carcinoma than gingival fibroblasts [128], and that it was toxic to leukemia cells but not to normal hematopoietic progenitors [129] or peripherical blood lymphocytes [130].

The regulation of p53 by resveratrol has been proposed to occur via activation of MAPKs (specifically ERKs and p38) [131]. Mitogen-activated protein kinases family members are implicated in cellular proliferation and also in apoptosis [132]. Resveratrol activated p38 and inhibited JNK in stimulated human cervical cancer cells [60], and it activated ERK1/2 in human breast cancer cells [62], human erythroleukemic cells [101] and human melanoma [133].

Moreover, p53 activity was shown to be regulated by resveratrol via modulation of p300 expression [54,127]. p300 is a transcription coactivator and member of the CREB-binding proteins family; it has acetyl-transferase activity that structurally alters many transcription factors, thereby exerting an antitumor role [134]. Targets of p300 include transcriptional factors known to be regulated by resveratrol, such as NF-kB, p53 [135] and Egr1 [136]. It is possible that p300 and p53 belong to an apoptotic loop of gene regulation that mediates resveratrol-induced apoptosis.

The retinoblastoma gene (Rb) product plays an important "gate keeper" role in the G1/S transition in the normal homeostatic situation. In the hypophosphorylated active form, Rb associates with E2F family of transcriptional factors, repressing gene transcription. By the end of G1 phase, hypophosphorylated Rb becomes hyperphosphorylated and inactivated by CDK/cyclin complexes [137], releasing E2F proteins. E2F complexes stimulate the transcription of genes that promote entry into S phase (including survivin, another target of resveratrol, as will be discussed later). Resveratrol action on Rb phosphorylation is controversial. In rat vascular smooth endothelial cells, high resveratrol concentration (100 μM) increased Rb phosphorylation and favored the transition into phase S but reversibly arrested the cell cycle in this phase, possibly via p53, although cellular levels of cyclin inhibitors p21 and p27 decreased [102]. Conversely, prolonged treatment (24–48 h) of human epidermoid carcinoma cells with low concentrations of resveratrol (10 μM) decreased the phosphorylation of Rb and the expression of E2F proteins and their complex-forming partners DP proteins, by up-regulation of p21 and the block of related CDK/cyclin complex, leading to cell cycle arrest in G0/G1 [118]. Interestingly it has been recently demonstrated that accumulation of free E2F may favor apoptosis by stabilizing and thus increasing p53 [138].

Another mechanism involved in resveratrol block of the cell cycle is related to its inhibition of DNA synthesis,

thereby impairing the normal course of the S phase. In particular, resveratrol inhibited ribonucleotide reductase activity in leukemia cells [139] and inhibited DNA polymerase activity in vitro [140].

Indeed, resveratrol ability to potentiate the effectiveness of apoptotic or cytostatic drugs requires pre- or cotreatment, whereas the addition of resveratrol after stressing stimulus is ineffective [109,141]. In vitro experiments demonstrated that resveratrol inhibited DNA polymerase, protein kinase D (PKD), cPKCs and COX2, although the concentration of the compound used in these in vitro assays was much higher than the tissual/cellular concentration available with dietary assumption or pharmacological treatment of resveratrol.

4.5.2. Cell survival-related proteins

Resistance to apoptosis may depend on intracellular levels of prosurvival and proapoptotic factors such as members of the Bcl2 family and the inhibitors of apoptosis (IAPs) proteins family.

The Bcl2 family of proteins includes pro- and anti-apoptotic factors. Proapoptotic proteins activate caspases, and this event is prevented by heterodimerization of proapoptotic with antiapoptotic proteins. Heterodimer formation is regulated by phosphorylation [142]. Overexpression of Bcl2 family proteins impairs resveratrol-induced apoptosis in T-acute lymphoblastic leukemia [143] and significantly attenuated resveratrol-induced apoptosis in monocytic leukemia [144], by impairing alterations in mitochondrial membrane permeability, production of radical oxygen species (ROS) [143], cytochrome C release and caspases activation [144].

Recently, it has been shown that natural polyphenols (e.g., catechins, epigallocatechins, theaflavins) bind Bcl2 and BclXl; the binding impaired the ability of these proteins to balance proapoptotic family members and thus it induced apoptosis [145]. Given structural similarities between these polyphenols and resveratrol, it will be interesting to study whether resveratrol is also able to interact with and sequester antiapoptotic factors. In colon carcinoma cancer cells, resveratrol induced mitochondrial translocation of proapoptotic Bcl2 family members (e.g., Bax, Bak) and initiated an apoptotic cascade [146]. Moreover, resveratrol treatment down-regulated the expression of antiapoptotic and increased that of proapoptotic Bcl2 members [127,146].

Survivin, a member of the IAP family, directly inhibits apoptosis, and its expression is frequently high in cancer cells and correlates with resistance to chemotherapy [147,148]. The survivin gene contains a cyclin-dependent element, and derepression of this element allows the expression of survivin at transition phase G1/S; survivin levels remain high during mitosis since it participates in chromosome assembly at the mitotic spindle [149]. Survivin expression is regulated by the formation or dissociation of different Rb/E2F complexes throughout the cell cycle [150], and it is switched off by an increase in p53/p21 complexes [151]. Besides regulating mitosis progression, survivin has a role in preventing apoptosis, possibly by impairing caspases activation and mitochondrial dysfunction [152].

Resveratrol decreased survivin levels by enhancing its degradation as well as reducing its transcription; this was associated with decreased proliferation and sensitization to chemotherapy [153]. Conversely, other authors assessed that resveratrol did not alter survivin or Bcl2 expression but it down-regulated other IAPs (cIAP1 and 2) related to apoptosis induction in a concentration-dependent manner [144].

4.5.3. Genomic regulation of cell cycle and apoptosis

NF-kB is a transcription factor involved in titration of the balance between proliferation and apoptotic stress response. The RelA/NF-kB family includes several proteins that, after release from cytosolic inhibitors (I-kB) via phosphorylation, translocate to the nucleus. Dimeric transcriptional factors are activated by serine phosphorylation due to PKA, MAPKs or PKCζ [154,155] and by acetylation due to different acetylases (including the CREB-binding protein, p300) [156]. Acetylation is a dynamic process that is reversed by deacetylases. Deacetylation reduces NF-kB transcriptional potential and increases its affinity for the cytosolic inhibitor I-kB [156]. The subset of activated genes depends on the combination of different monomers forming active NF-kB as well as on combinatorial interactions with promoter-bound factors, according to stimuli and cell type [157].

AP1 is another transcriptional factor produced by a variety of dimeric combinations of proteins of the Jun and Fos families. AP1 is regulated via phosphorylation by MAPKs and interacts with other factors such as NF-kB, CBP/p300 and Rb, thus regulating target genes common to NF-kB. Like NF-kB, AP1 is also considered to be a proliferation and tumor growth promoter [158].

Resveratrol was shown to inhibit NF-kB activation; with some exceptions [159,160], this activation was associated with an antiproliferative action and with the induction of cell death [104,126,127]. NF-kB controls the transcription of a variety of genes, including tumor-promoting COX2, iNOS, matrix metalloprotease (MMP9) and endothelial adhesion molecules [157]. The expression of these genes was reported to be down-regulated by resveratrol in different cell lines [60,85,86,98]. In addition, dietary administration of resveratrol in DMBA-induced tumor-bearing rats reduced tumor growth and decreased transcription of NF-kB and of its regulated genes COX2 and MMP9 in tumor tissues [98]. Resveratrol was shown to inhibit NF-kB, AP1 and their target genes regulating the activity of the upstream MAPKs. Resveratrol down-regulated TNFα induction of NF-kB by blocking JNK and MEK activation [161], and it down-regulated phorbol myristate acetate (PMA)-induced NF-kB by blocking JNK and PKCδ activation [60]. Similarly, resveratrol down-regulated AP1 induction by PMA and UV irradiation, possibly by upstream inhibition of Src and MAPKs [61].

Resveratrol inhibition of tyrosine kinases such as Raf [162] and Src [61] may be considered an upstream event that

opens access to multiple cascades. Studies either confirmed [61] or refuted [23] resveratrol inhibition of Src activity in vitro, questioning the possibility of a direct action.

The potential of resveratrol as an antiangiogenic molecule can be related to its inhibition of NF-kB activation. In TNFα stimulated endothelial cells, resveratrol impaired NF-kB activation only after prolonged and not acute treatment [163]. Moreover, low doses (1 μM) of resveratrol blocked Src activation upon growth factor stimulation of human umbilical endothelial cells [23].

Another proposed mechanism for resveratrol inhibition of NF-kB is via impairment of phosphorylation and activation of PKD. In vitro assays revealed that resveratrol inhibited, although weakly (IC_{50} 100 μM), PKD autophosphorylation, which is necessary for the kinase activation [164]. Protein kinase D is activated by Src- and Abl-mediated tyrosine phosphorylation and by PKCä serine phosphorylation; PKD activation is upstream to the NF-kB survival response to oxidative stress [165]. Resveratrol inhibited PKC activity [61,166,167]. Some authors reported that resveratrol has a selective inhibitory effect on PKCä [60], and therefore, it can impair both PKD and downstream NF-kB activation [168]. It is still unclear if resveratrol affects directly PKCδ activity, since the evidence of resveratrol competition for phorbol ester binding domain (IC_{50}, 2 μM) [167] and kinase domain (IC_{50}, 90 μM) [169] has been obtained studying, respectively, classic PKC isoforms or an isozymes mixture from rat brain.

Recently, it has been demonstrated that resveratrol is a powerful activator of sirtuins transcription and function [170]. Sirtuins are a nicotinamide adenosine dinucleotide (NAD)-dependent class of deacetylases responsible for regulating the response to DNA damage and gene silencing processes of aging and survival [171,172]. Resveratrol activated human sirtuin 1 (SIRT1) and this mediated RelA/p65 deacetylation, thus inhibiting TNFα-induced NF-kB transcription and sensitizing cells to apoptosis [173].

This evidence contrasts with the observations that SIRT1 deacetylated and inactivated p53 [174] in a concerted and still not defined equilibrium with the action of histone deacetylase HDAC1 [121], thus providing a possible mechanism of blocking apoptosis and promoting cell survival [121,175]. In addition, resveratrol enhanced the expression p300 [54], an acetylase that activates NF-kB [135,156] and p53 [135]. In this sense, Yeung et al. [173] showed that resveratrol failed to inhibit and actually increased TNFα induction of NF-kB when SIRT1 was pharmacologically inhibited, suggesting that its activity may mediate resveratrol down-regulation of NF-kB; conversely, the overexpression of SIRT1 reversed TNFα-induced and possibly p300-mediated activation of NF-kB.

From the reported data, it is possible to speculate that resveratrol controls two key gene transcriptional regulators: p300 and SIRT1. It remains to be assessed whether this action could be a direct one by increasing enzyme activity, as has been shown in vitro for sirtuin [170]. Both p300 and SIRT1 activate transcriptional factors that trigger opposite pathways of apoptosis and proliferation. p300 activates (by acetylation) whereas SIRT1 inhibits (by deacetylation) both the proliferative NF-kB and the apoptotic p53. This hypothesis requires further investigation that may be important in elucidating a reported dual effect of resveratrol as an inducer of cell death as well as of proliferation (see following paragraph).

Egr1 is a transcriptional factor that activates genes related to cell growth and differentiation. Egr1 belongs to a group of early growth response genes (e.g., p53, Rb, growth factors, multidrug resistance MDR1, c-*jun*, c-*fos*). It is regulated by forming complexes with cofactors, such as CBP (p300) acetylases [136]. It is activated in response to cytokines, stress and cytotoxic agents and involved in proliferation and in cell death pathways [176,177]. Mitogen-activated protein kinases may be upstream mediators of Egr1 transcription [178,179].

Resveratrol was shown to activate Egr1. Egr1 can bind p21 promoter in vivo, and antisense Egr1 mRNA impaired resveratrol-induced p21 up-regulation [180]. Moreover, Egr1 transcription is mediated by ERKs, since specific inhibitors of these kinases block the resveratrol-induced increases in Egr1 [112,181]. It is interesting to note that resveratrol-induced early increase in the expression of Egr1 was not related to transcriptional events; conversely, a delayed and sustained expression of Egr1 resulted as a mediated event that was inhibited by cycloheximide [180]. This supports the hypothesis that Egr1 is a direct resveratrol target.

These observations suggest that cellular response to resveratrol involves gene transcription-mediated promotion of parallel or overlapping and potentiating cascades of events. By modulating upstream tyrosine and serine kinases, resveratrol may regulate the activation of transcriptional factors directed to clusters of genes responsible for inducing cell cycle arrest and eventually apoptosis. This hypothesis is summarized in Fig. 6.

4.5.4. Sphingolipid signaling

Ceramide is a sphingolipid mediator of intracellular signals, normally present in membranes in a complexed form as sphingomyelin or gangliosides. In conditions of stress and aging, there is an increased production of ceramide by de novo synthesis and release by hydrolysis of complex sphingolipids. Ceramide interacts, either directly or indirectly, with a variety of intracellular targets, leading to differentiation, cell cycle arrest and apoptosis.

Recently, resveratrol was shown to promote intracellular accumulation of ceramide in breast and prostate cancer cells [182,183]. Resveratrol enhanced the de novo synthesis of this sphingolipid by increasing the activity of the rate-limiting enzyme. Moreover, inhibitors of ceramide formation (enzyme blockers) rescued cells from resveratrol-induced apoptosis. This observation identified a new important checkpoint in the actions of resveratrol. Although this polyphenol triggers multiple pathways, ceramide production

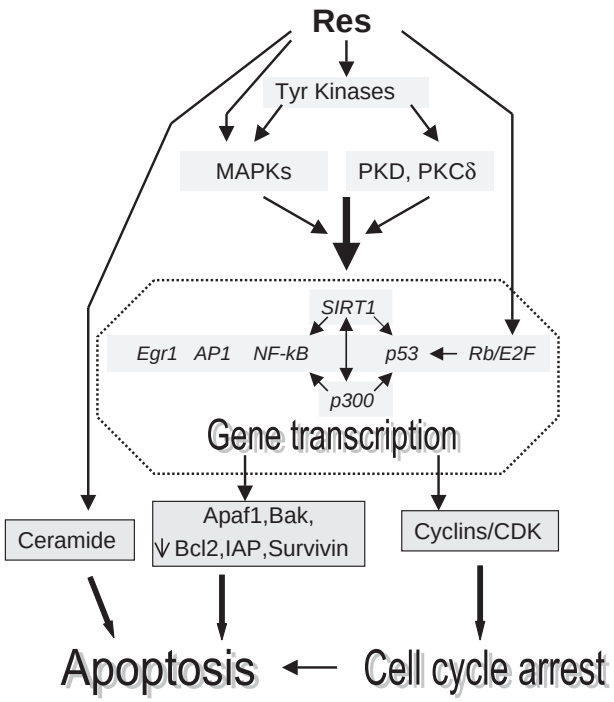

Fig. 6. Pathways in which resveratrol modulates cell cycle arrest and apoptosis.

may be a common step that drives cells toward irreversible death (Fig. 6).

4.5.5. Drug transporters

In addition to interfering with cell cycle control, resveratrol affects multiple targets that are involved directly or indirectly in apoptotic pathways and chemotherapy responsiveness. An intriguing hypothesis is that resveratrol, like other flavonoids, is able to overcome drug resistance of tumors that express multidrug resistance-associated proteins (MRP). These ATP-dependent pumps efflux chemotherapeutics out of cells [184].

Resveratrol was reported to interact with the breast cancer resistance protein (BCRP): it competed with other substrates for BCRP, impairing its activity and caused the intracellular accumulation of drugs [185]. It is important to note, however, that BCRP is also normally expressed in placenta in order to protect the fetus from maternal blood estrogens and at the luminal surface of the blood–brain barrier.

5. Effects of resveratrol on tumoral versus normal cells

A few reports have offered an interesting perspective on resveratrol actions on normal versus malignant cells. One study that compared different leukemia cell lines and bone marrow progenitor cells found that the IC_{50} of resveratrol for proliferation inhibition varied almost twofold: 34 μM in leukemia and 59 μM in hematopoietic cells. Moreover, in a colony formation assay performed after a pulse of resveratrol (80 μM for 20 h), there was a significant difference in the ability to proliferate between hematopoietic progenitors and leukemia cells. Human fibroblasts transformed with SV40 virus (WI38VA) were sensitive to resveratrol modulation of pro- versus antiapoptotic genes whereas normal fibroblasts were not [186].

Not only in leukemia but also in breast cancer, resveratrol induced apoptosis via CD95-dependent caspase activation (anti-CD95 antibody rescued from apoptosis). Both tumors constitutively expressed CD95, and resveratrol promoted C95-ligand expression, which acted as an autocrine apoptosis inducer. Interestingly, peripheral blood lymphocytes, although CD95-positive, did not respond to resveratrol by ligand expression and therefore were insensitive to the compound cytotoxicity [130]. A slight difference in sensitivity to resveratrol has been reported between tongue squamous carcinoma cells and normal gingival fibroblasts [128], possibly related to the lower proliferation rate (therefore, higher resistance) of normal cells versus immortalized or highly proliferating tumoral cells.

These promising results contrast with those of other studies in which resveratrol exhibited similar effects on normal and neoplastic cells. Comparable cytostatic and cytotoxic potentials have been observed in breast cancer cell lines and in immortalized mammary epithelial cells [51].

6. Resveratrol double identity: prosurvival versus prodeath

What is particularly intriguing about resveratrol is its dual action. In plants, resveratrol is an antioxidant, protecting against damage induced by light exposure. However, its overproduction, triggered by excessive stress, can be considered as a hypersensitivity reaction that may lead to cell death. Considering the bioactivity of resveratrol in humans, dietary assumption may impair tumorigenesis by inhibiting the formation of toxic cellular metabolites and enhance the activity of sirtuins known to be related to cell survival. However, resveratrol is also able to arrest cell cycle and to induce apoptosis in many human tumor cell lines as well as in animals in vivo. What is the thread that connects these two signaling pathways that are commonly considered as diverging cascades?

Being a phytoestrogen, resveratrol activated steroid receptor-mediated proliferative pathways, as has been shown in osteoblasts [187] and breast cancer cells [48]. Nonetheless, antiproliferative effects have been shown to occur independently of ER expression or transcriptional activity [47].

NF-kB and AP1 are activated in response to a variety of proliferative or stress signals [188]. Resveratrol inhibits NF-kB activation and proliferative responses, as previously discussed. In addition, Manna et al. [59] reported that resveratrol inhibited NF-kB activation in human myeloid lymphoma in response to apoptotic stimuli such as LPS and ceramide, therefore, impairing caspases activation and apoptosis. Resveratrol blocked p65 subunit phosphorylation

and nuclear translocation, thereby inhibiting NF-kB-mediated activation of JNK and phosphorylation of MEK. It is to note that at least 4 h of preincubation with resveratrol is required for this effect [59].

Pretreatment with low doses (4–8 μM) of resveratrol for 2–4 h prevented H_2O_2-induced apoptosis [59,189]. Reactive oxygen species, including superoxide O_2^- and H_2O_2, are maintained at nontoxic concentrations by intracellular antioxidant systems. If on one hand high concentrations of ROS create a necrotic stress, a slight increase may represent instead a proliferative stimulus. This often becomes an acquired survival and growth-promoting mechanisms in cancer cells [190]. It has been demonstrated that a moderate increase in O_2^- counteracts cell death and that the apoptotic potential of H_2O_2 is strengthened by its abilities to decrease the intracellular concentration of O_2^- and to reduce cytosolic pH [191,192]. Human leukemia cells, pretreated for 2 h with resveratrol at low doses, exhibited a significant increase in intracellular O_2^- that impaired H_2O_2-induced acidification, caspases activation and apoptosis [189].

In 2003, Howitz et al. [170] observed, in a yeast model, that resveratrol activated sirtuin (SIRT2, yeast homologue of human SIRT1). In yeast, sirtuins are activated in response to calorie restriction, which prolongs the cell life span. Resveratrol effects on sirtuins have also been studied in animal and human models. In HEK 293 cells, low doses of resveratrol (0.5 μM) partially protected from radiation-induced apoptosis; this was associated with deacetylation and inactivation of p53 due to SIRT1 activation, although this protection effect was reversed when cells were treated at higher concentrations (50 μM) [170]. Resveratrol directly regulated the activity in vitro and lowered the Km of recombinant human SIRT1 for its acetylated substrate and for NAD^+ [170]. In rat adipocytes, resveratrol (50–100 μM) stimulated lipolysis and fatty acid mobilization from triglycerides, probably by binding DNA and preventing peroxisome proliferator-activated receptor (PPARγ)-induced gene transcription [193]. Elongation of the life span in yeast and protection from cell death or white fat mobilization in mammalian cells represent responses to stress mediated by sirtuins. Resveratrol may mimic this stimulus and activate sirtuin-mediated intracellular events in order to counteract the stressing agent and allow the cell to live. On the other hand, dietary restriction inhibits signaling pathways (e.g., involving MAPKs and AP1) related to proliferation and cancer promotion [194,195]; similarly, resveratrol affects these targets in a variety of human tumors by acting as an antitumor agent.

These observations suggest that resveratrol has different effects according to cellular conditions, specific cell molecular settings and finally the concentration used. Resveratrol up-regulated the expression and/or enhanced the activity of transcriptional regulators: acetylase-transferases such as p300 and deacetylases such as sirtuin. Each of these two may exert opposite effects, as discussed previously. In this scenario, it is possible that in cells with altered metabolism and unbalanced expression of proliferative over apoptotic regulators or subjected to stress factors (such as chemotherapeutics or cytokines), the equilibrium between the two opposite pathways results unbalanced thus particularly sensitive to resveratrol. Moreover, epigenetic regulation of gene transcription (DNA methylation and histone methylation, phosphorylation, acetylation and ubiquitination) is frequently associated with cancer promotion and progression [196], and the activity of deacetylases, by targeting transcriptional factors among other substrates, contributes significantly to cell cycle regulation [197]. Resveratrol modulation on acetylation/deacetylation mechanism requires further investigation to define applicable dose and tumor responsiveness and it might to turn out to be an upstream event that promotes the transcription of those sets of genes responsible for triggering signaling cascades.

7. Conclusions

In the last decade, the number of studies on resveratrol has dramatically increased from five publications in 1993 to over 200 in 2003. This is not surprising, as resveratrol is interesting for its participation in both prosurvival and prodeath cellular mechanisms, favoring the preservation of the functional status of cells and possibly elongating a cell life span, and inducing the death of cells whose physiological conditions have become deranged. Therefore, an increasing number of researchers are joining the challenge to unravel the mysteries of this fascinating and promising molecule, hopefully with success.

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