

Chemoprevention by resveratrol: molecular mechanisms and therapeutic potential

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1. ABSTRACT

Resveratrol, a polyphenol found in numerous plant species, including mulberries, peanuts and grapes, has shown to possess chemopreventive properties against several cancers, and cardiovascular diseases. Recently, resveratrol has been shown to have positive effects on age longevity, lipid levels and a preventative quality against certain cancers and viral infections. Resveratrol induces apoptosis by up-regulating the expression of Bax, Bak, PUMA, Noxa, Bim, p53, TRAIL, TRAIL-R1/DR4 and TRAIL-R2/DR5 and simultaneously down-regulating the expression of Bcl-2, Bcl-X_L, Mcl-1 and survivin. Resveratrol causes growth arrest at G1 and G1/S phases of cell cycle by inducing the expression of CDK inhibitors p21^{WAF1/CIP1} and p27^{KIP1}. Resveratrol has also been shown to reduce inflammation via inhibition of prostaglandin production, cyclooxygenase-2 activity, and nuclear factor-

κB activity. Modulation of cell signaling pathway by resveratrol explains its diverse bioactivities related with human health. Resveratrol also potentiates the apoptotic effects of cytokines, chemotherapeutic agents and gamma-radiation. Pharmacokinetic and pharmacodynamic studies demonstrated that the main target organs of resveratrol are liver and kidney, and it is metabolized by hydroxylation, glucuronidation, sulfation and hydrogenation. As a chemoprevention agent, resveratrol has been shown to inhibit tumor initiation, promotion, and progression. There is growing evidence that resveratrol can prevent or delay the onset of various cancers, heart diseases, ischemic and chemically induced injuries, pathological inflammation and viral infections. This review summarizes the molecular mechanisms of resveratrol and its clinical benefits for human diseases.

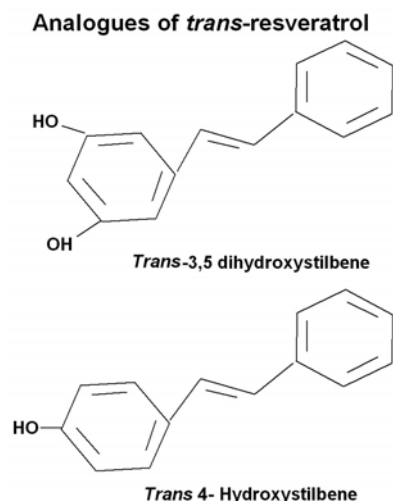


Figure 1. Analogues of *trans*-resveratrol.

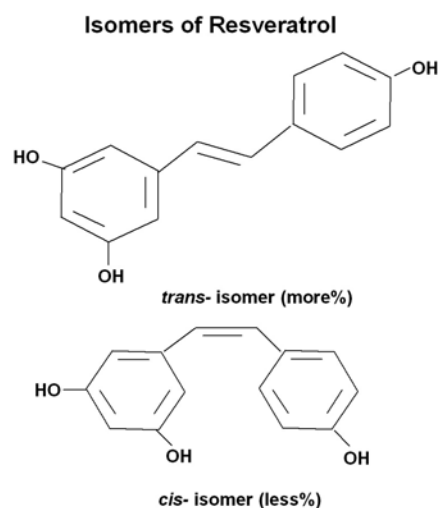


Figure 2. Structure of resveratrol both *cis*- and *trans*-isomers.

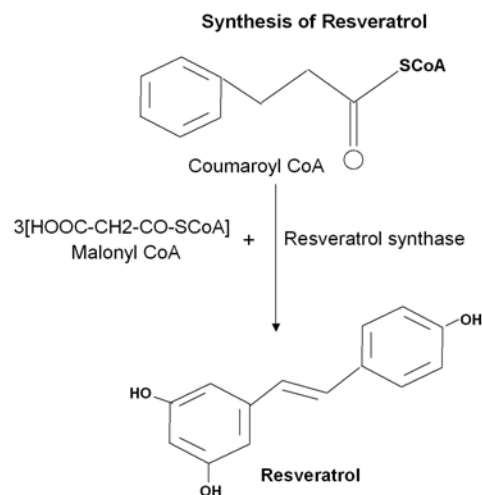


Figure 3. Synthesis of resveratrol.

2. INTRODUCTION

Resveratrol (C₁₄H₁₂O₃) is a naturally occurring phytoalexin produced by some higher plants in response to stress/injury due to environmental hazards. Phytoalexins are chemical substances produced by plants as a defense against infection by pathogenic microorganisms such as fungi. The polyphenolic compound resveratrol, which is also known as 3,4',5 trihydroxystilbene and 3,4',5-stilbenetriol (Figure 1), exists in *cis*- and *trans*-stereoisomeric forms commonly found in grapes and wines particularly red wine (Figure 2). *Trans*-resveratrol can undergo isomerization to the *cis*-form when heated or exposed to ultraviolet irradiation. It is synthesized from p-coumaroyl CoA and malonyl CoA (Figure 3). It is a naturally occurring phytochemical found in many plant species, including grapes, peanuts and various herbs and it is the parent molecule of a family of polymers called viniferins.

The age-adjusted incidence of cancer has been increasing by approximately 3% annually worldwide (1). Many risk factors for human cancer have been proposed, including genetic predisposition, age, diet, hormones, and environmental factors. However, its etiology is still largely unknown. The importance of prevention in reducing the morbidity and mortality from cancer has been widely recognized. A promising avenue for future clinical chemoprevention studies focuses on phytochemicals/phytoalexins, as a means of cancer preventive compounds, found in fruits, vegetables and other plants. Many phytochemicals are excellent potential chemopreventive agents, because, in addition to their cancer preventive effects, they are relatively non-toxic, natural and inexpensive, they can be taken orally and may have additional health benefits. New opportunities in clinical chemoprevention research include investigating the chemopreventive effects of phytochemicals and conducting studies in patients with cancer. There is also a great need to investigate potential benefits and risks of administering phytochemicals/natural compounds before, during or after conventional therapies, such as surgery, chemotherapy, radiation or hormonal therapy. In addition, administration of chemo preventive agents prior to surgery provides an opportunity to investigate the modulation of genetic and epigenetic pathways by putative cancer preventive compounds and nutrients.

While many new classes of cancer chemopreventive agents are being evaluated in clinical trials for other malignancies, little success has been achieved so far. Chemoprevention of cancer is a means of cancer control where the occurrence of disease can be entirely prevented, slowed or reversed by the administration of one or a combination of naturally occurring or synthetic compounds (2, 3). The overall goal of this modality is to reduce cancer incidence and multiplicity in the first place. The chemopreventive compounds are also known as anticarcinogens where the preventive approach includes intervention (or secondary prevention) of the conversion of precancerous lesions into malignant carcinomas (3).

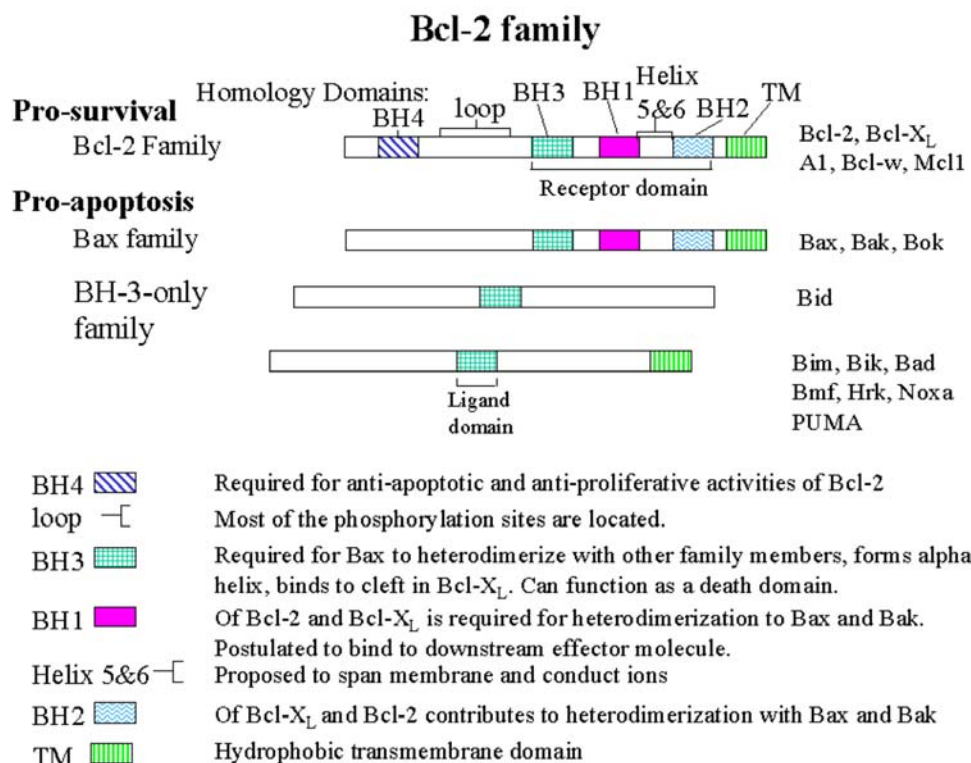


Figure 4. Structure of Bcl-2 family members. Bcl-2 family contains both anti-apoptotic (Bcl-2, Bcl-X_L, A1, Bcl-w and Mcl-1) and pro-apoptotic (Bax, Bak, Bok, Bid, Bim, Bik, Bad, Bmf, Hrk, Noxa and PUMA) proteins. Known α -helical regions are indicated. BH (BH1-4) domains are highly conserved among family members. Most members have a carboxy-terminal hydrophobic domain that aids association with intracellular membranes, the exception being A1 and many of the BH-3 only proteins (Bad, Bid, Noxa, Bim and Puma).

Resveratrol has been shown to have anti-inflammatory, antioxidant, antitumor, neuroprotective, and immunomodulatory activities (4-6). It also has activity in the regulation of multiple cellular events associated with carcinogenesis. Resveratrol has also been examined in several model systems for its potential effect against cancer (7-9). Its anticancer effects include its role as a chemopreventive agent, its ability to inhibit cell proliferation, its direct effect in cytotoxicity by induction of apoptosis and on its potential therapeutic effect in pre-clinical studies (10, 11). It induces growth arrest and apoptosis through regulation of multiple signaling pathways. The purpose of this review is to summarize the molecular mechanisms of resveratrol and assess its therapeutic potential for human diseases.

3. MECHANISM OF ACTION

3.1. Regulation of Bcl-2 family members

The Bcl-2 proto-oncogene was discovered at the chromosomal breakpoint of t(14;18) bearing human B-cell lymphomas. The Bcl-2 family of proteins has expanded significantly and includes both pro- as well as anti-apoptotic molecules (Figure 4). Some of the members of this family (e.g. Bcl-2 and Bcl-X_L) inhibit apoptosis, and while others (e.g. Bcl-X_S, Bax and Bak) promote apoptosis (12-15). Indeed, the ratio between these two subsets helps

determine, in part, the susceptibility of cells to a death signal (16). An additional characteristic of the members of this family is their frequent ability to form homo- as well as hetero-dimers, suggesting neutralizing competition between these proteins. Furthermore, these members have ability to become integral membrane proteins and regulate several physiological events.

Bcl-2 family members possess up to four conserved Bcl-2 homology (BH) domains designated BH1, BH2, BH3, and BH4, which correspond to α -helical segments (17-19). Many of the anti-apoptotic members display sequence conservation in all four domains. The pro-apoptotic molecules frequently display less sequence conservation of the first α -helical segment, BH4. Deletion and mutagenesis studies argue that the amphipathic α -helical BH3 domain serves as a critical death domain in the pro-apoptotic members. This concept is supported by an emerging subset of "BH3-domain-only" members who display sequence homology only within the BH3 domain and to date are all pro-apoptotic. However, the three-dimensional structure of at least one BH3-domain-only molecule, BID, demonstrates a very similar overall α -helical content to the anti-apoptotic molecule Bcl-X_L (20). Many Bcl-2 family members also contain a carboxy-terminal hydrophobic domain, which in the case of Bcl-2 is

essential for its targeting to membranes such as the mitochondrial outer membrane. Overexpression of Bcl-2 and Bcl-X_L protein protects a wide variety of cells from many death-inducing stimuli (21-24). Resveratrol inhibits the expression of antiapoptotic proteins such as Bcl-2 and Bcl-X_L, and induces the expression of Bax, Bak, Bad, PUMA, Noxa and Bim (25-30). These data suggest that the regulation of Bcl-2 family members plays a major role in resveratrol-induced apoptosis.

3.2. Regulation of Cell Cycle

Resveratrol has been reported to modulate cell cycle and to induce apoptosis. Several authors have studied the effect of the stilbene on cell cycle-control. In colon cancer cells, a down-regulation of the cyclin D1/Cdk4 complex has been reported (31), while in transplantable liver cancer H22 cells, resveratrol decreased cyclin B1 and Cdc2 protein, although no alteration of cyclin D1 was observed, G₂ arrest was itself been reported to be linked with the inhibition of Cdk7 and Cdc2. In addition, an S-phase arrest was also been noticed in melanoma cells, being related to cyclins A, E, and B1 (32). Thus, it is clear that the effects of resveratrol on cell cycle are highly variable, depending on the cell line observed. An additional complexity level occurs, related to a dose-dependent action of resveratrol on DNA synthesis (32), and attributed to the modulation of nuclear p21^{Cip1/WAF1} and p27^{Kip1} levels. Our data indicate a decrease (at nanomolar resveratrol concentrations) of DNA synthesis, and therefore a decrease of cells entering S-phase, suggesting that the p21 pathway may not be involved in its action. However, the observed accumulation of cells in G₁ could also imply the Rb or the p53 pathways. Indeed, it was shown that resveratrol treatment of melanoma cells resulted in reduced hypophosphorylated Rb and an increased hypophosphorylated Rb. This response was accompanied by down-regulation of the expression of all five E2F family transcription factors and their heterodimeric partners DP1 and DP2, introducing an arrest of cell-cycle progression at the G₁/S phase transition, thereby leading to subsequent apoptotic cell death. In addition, in melanoma (33), endothelial (34) and fibroblastic cell lines (35), resveratrol treatment led to an activation of p53 activity, which correlated with suppression of cell progression through the S and G₂ phases of the cell-cycle and apoptosis. The effect of resveratrol on the G₂-phase of cell cycle could be due to the action of resveratrol on the cytoskeleton (36). Catechin oligomers, acting on membrane androgen receptors could, induce apoptosis, through actin filaments rearrangement. Overall, these data suggest that resveratrol causes growth arrest at G₁, G/S and G₂ phase of the cell cycle.

3.3. Regulation of Mitogen Activated Protein Kinase

Mitogen-activated protein kinase (MAPK) pathways are now well understood and it is well presented in mammalian signal pathways and includes p38, c-Jun N-terminal protein kinase (JNK), and extra cellular signal-regulated kinase (ERK). These MAP kinase pathways consist of several other kinases, which activate each other via phosphorylation cascades and thus activate several transcription factors serially (37, 38). The JNK and p38 pathways have showed their relation with apoptosis, while

ERK was shown to effect cellular proliferation and differentiation. The interaction between these MAP kinases pathways and resveratrol provides future beneficial therapies regarding its anticancerous property. As it has already been shown that Resveratrol has its effect on upregulating p53 protein thereby downregulating the expression of NF- κ B and AP-1 simultaneously (39). In previous studies, it has been proven that at least half of the human cancers occur due to loss or/mutation of this p53 protein (39). In fact resveratrol has found to induce apoptosis in mouse fibroblast cells with wild-type p53, but could not work out in absence of p53-protein in mouse fibroblast cells (39). Studies have shown that resveratrol-induced apoptosis by activating p53 expression (via phosphorylation) mediated through ERK and p38 pathways (37, 38). Similarly, resveratrol downregulates the expression of AP-1 which is thought to be the key factor in inducing melanoma in humans.

3.4. Generation of Reactive Oxygen Species (ROS) by Resveratrol

Reactive oxygen species (ROS) include free oxygen ions, free radicals and both inorganic as well as organic peroxides. They are highly reactive due to presence free electrons and are generally very small molecules. ROS are formed during natural metabolism as a byproduct and have important roles in cell signaling (40). However, during times of environmental stress ROS levels can dramatically increased, which can result in significant damage to cell structures due to presence of nascent molecule. This aggregates into a situation known as oxidative stress. Cells are normally able to defend themselves against ROS damage through the use of enzymes such as superoxide dismutases (SOD) and catalases. Small molecule antioxidants such as ascorbic acid (vitamin-C), uric acid, and glutathione also play important roles as cellular antioxidants. Similarly, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals through these SOD pathways. At low levels, ROS such as superoxide, hydrogen peroxide, and hydroxyl radical, may function in cell signaling processes, whereas at higher levels, ROS may damage cellular macromolecules (nucleic acids) and participate in apoptosis (40, 41). ROS have been implicated as a key factor in the activation of p53 by many chemotherapeutic drugs. ROS-mediated disruption of $\Delta\psi_m$ constitutes a vital step in the apoptotic pathway of p53. Apoptosis triggered by p53 has been reported to be dependent on an increase in ROS and the release of apoptotic factors from mitochondrial damage (42, 43). These studies suggest that ROS are downstream mediators in p53-dependent apoptosis in transcription-dependent or transcription-independent pathways. When cells are exposed to oxidative stress, p53 is expressed at high levels by posttranslational modifications, including phosphorylation, acetylation, and glycosylation (44, 45). These events occur rapidly and lead to the activation of p53, resulting in either cell cycle arrest or apoptosis. These findings suggest the novel functions of ROS as p53 activators or p53 downstream effectors.

Excessive NO production results in limited angiogenesis and, in some tumor cells, increased apoptosis,

while lower amounts can increase vascularity and protect cells from apoptosis. In normal liver, hepatocytes express low levels of endothelial and inducible NOS. Nevertheless, during liver injury, there is a substantial increase in endothelial NOS, followed by induction of the inducible form of the enzyme; the subsequent massive NO production was incriminated to trigger the onset of several hepatopathies. Hence, it has been suggested that NO may possess a dual pro- and anti-tumor activity, depending on the local concentration of the molecule.

Resveratrol has been reported to increase as well as decrease NO production (46, 47). The involvement of NOS has already been reported in cultured pulmonary artery endothelial cells, and gastric adenocarcinoma cells (48), leading to inhibition of their proliferation. Indeed, inhibition of NOS activity reverted resveratrol action, indicating a direct relationship of increased NO production and inhibition of cell growth. In addition, resveratrol has been shown to modify iNOS expression (49-52).

3.5. Regulation of PI-3-K/AKT Pathway

Loss of chromosome 10q is a frequently observed genetic defect in prostate cancer. The PTEN/MMAC tumor suppressor gene was identified and mapped to chromosome 10q23.3. PTEN encodes a protein/lipid phosphatase (53), which is mutated or deleted in a range of human cancers including prostate (54-57). Recent work has indicated that loss of PTEN function occurs in 10-20% of organ-confined and over 50% of advanced prostate cancers, placing it among the most common molecular abnormality reported in human prostate cancer. Notably, knockout mice lacking PTEN develop multiple cancers, including prostatic hyperplasia and prostatic intraepithelial neoplasia. This is supported by recent observation showing a correlation between the status of PTEN and activation of the serine/threonine kinase Akt. Thus, PTEN functions as a negative regulator of the PI3-kinase/Akt-signaling pathway.

Through direct regulation of PIP3 levels, PTEN negatively regulates the PI3K signaling pathway, which transduces extracellular growth regulatory signals to intracellular mediators of growth and cell survival (58). Accordingly, in tumors, inactivating mutations in PTEN led to increase activity of Akt/PKB, one of the most well-characterized downstream effectors of PI3K (59, 60). Because PI3-kinase and Akt are known to have oncogenic and/or antiapoptotic functions in various model systems, it is likely that deregulation of this pathway in PTEN-deficient cells is responsible for the cancer phenotype. Future work delineating the role of this signaling pathway using genetically defined mouse models for prostate cancer, and on testing the therapeutic role of drugs that target this pathway could be very beneficial. Thus, loss of PTEN (or higher Akt activity) may be one of the major factors involved in TRAIL resistance.

Akt/PKB is activated in response to activation by many different growth factors (GFs) (61). Akt/PKB is the cellular homologue of the product of the *v-akt* oncogene (62, 63) and has three isoforms: Akt-1, -2, and -3. Activation of all three isoforms is similar in that

phosphorylation of two sites, one in the activation domain and one in the COOH-terminal hydrophobic motif, are necessary for full activity. Once activated, however, Akt/PKB exerts antiapoptotic effects through phosphorylation of substrates such as Bad (64, 65) or caspase-9 (66) that directly regulate the apoptotic machinery, or substrates such as the human telomerase reverse transcriptase subunit (67), forkhead transcription family members (68, 69), or IB kinases (70) that inhibit apoptosis indirectly (71). Phosphorylated Bad has low affinity to Bcl-X_L and high affinity to the 14-3-3 proteins, thus activating its proapoptotic function (72, 73). Furthermore, Akt plays an important role in the stimulation of cell proliferation by inactivating cyclin-dependent kinase inhibitor p21^{WAF1/CIP1} and decreasing the transcription of p27^{KIP1} (74). Akt is an important regulator of cell proliferation, cell survival, and significantly contributes to tumor growth and progression by promoting cell invasiveness and angiogenesis (75-81). Overexpression of Akt has been reported in a variety of human cancers including prostate cancer (61, 82), and cells expressing elevated levels of Akt are less sensitive to apoptosis stimuli (74). An increase in Akt activity has been linked with prostate tumor progression and the development of androgen-independent prostate cancer (83). We have recently demonstrated that Akt is activated in the LNCaP cell line (84). LNCaP cells use Akt for survival because when PI3-K inhibitors are added or kinase-dead Akt is transfected, LNCaP cells undergo apoptosis (84). These data suggest that targeting a specific kinase that promotes survival such as Akt could change the apoptosis-inducing potential of TRAIL or resveratrol.

3.6. Regulation of Transcription Factors

NFκB family is a group of structurally related and evolutionary conserved proteins subunits that have been identified and cloned in mammalian cells (85). These include Rel (c-Rel), RelA (p65), RelB, NFκB1 (p50/p105) and NFκB2 (p52/p100). The NFκB subunits form homo- or hetero-dimers through their rel homology domain (RHD), which is also responsible for the DNA binding of NFκB and interaction of NFκB with IκB, the family of inhibitory proteins of NFκB (86). NFκB usually exists in a latent state in the cytoplasm and its activation requires extracellular stimuli leading to the phosphorylation and subsequent proteasome-mediated degradation of inhibitory IκB proteins (87). Finally, dimeric activated NFκB translocates into the nucleus and controls the expression of target genes. However, constitutively active NFκB was identified in a variety of cancers and linked to the oncogenesis and tumor resistance (88).

The involvement of NFκB in cancer has been originally suggested because of the homology of mammalian c-Rel with highly oncogenic retroviral v-Rel, which induces aggressive tumors in chicken (89, 90). In mammalian cells, NFκB also regulates expression of more than 150 genes and some of them were linked to cancer initiation, proliferation, angiogenesis, survival and metastasis (91). Interestingly, NFκB activation was detected before tumor initiation, therefore connecting

aberrant stimulation of expression of NF κ B-regulated genes prior to growth and progression of cancer (92).

Mitotic cellular division is a crucial feature in the growth of normal as well as cancer cells. The cellular division can be characterized as a cycling process where cells are proceeding from the resting stage (G_0) to DNA synthesis (S) and mitosis (M) stages of cell cycle. During the cell cycle the cells have to go through gap phases G_1 and G_2 , which are before and after DNA synthesis (S) phase. Cell division is conceived as a passage through a series of check-points with the earliest point late in G_1 , when cells become irreversibly committed to DNA synthesis and division (93, 94). The check-points are controlled by a group of D cyclins, and cyclins E and A. Cyclin D1 acts in G_1 and promote progression through the G_1 -S phase of the cell cycle in mammalian cells. The cyclin D1 protein is a regulatory subunit of holoenzyme forming a complex with catalytic subunits of cyclin-dependent kinases (cdks) cdk4 and cdk6, which phosphorylates and inactivates the retinoblastoma protein pRB (93, 94). The check-points of the cell cycle are usually deregulated in oncogenesis, and amplification or overexpression of cyclin D1 have been identified in the development of a subset of human cancers including cancers of breast (95, 96), prostate (97), lung (98), colon (99), bladder (100, 101), ovary (102), liver (103), pancreas (104), brain (105) and esophagus (105). Furthermore, overexpression of cyclin D1 has been identified in melanomas (106), sarcomas (107), parathyroid adenomas (108), myelomas (109) and myeloid leukemias (110). In addition to the regulation of expression by NF κ B, the cyclin D1 regulatory region also contains binding sites for activator protein (AP-1) (c-Jun/c-Fos) (111), STAT5 (112), E2F (113), Sp-1/Sp-3 (114), lymphoid enhancer factor-1 (LEF-1)/ β -catenin (113), and CREB/CREM proteins (115). However, the constitutive activation of NF κ B (and AP-1) is probably the major factor responsible for the overexpression of cyclin D1 in a variety of cancers. The role of cyclin D1 in tumorigenesis has been also confirmed by the overexpression of cyclin D1 in various experimental transgenic mice, which resulted in growth of tumors in the mammary glands (116). In human multiple myeloma cells, resveratrol inhibited constitutive activation of both NF κ B and STAT3, leading to down-regulation of cell proliferation and potentiation of apoptosis induced by bortezomib and thalidomide (29). These studies suggest that resveratrol may have a potential in the treatment and prevention of cancer.

3.7. Regulation of TRAIL-Death Receptor Pathway

TRAIL, belonging to the TNF cytokine family, is a type II membrane protein that induces apoptosis in a wide variety of transformed cells (117-120). Histological analysis of TRAIL-treated tumors revealed an increase in apoptotic cells and confirmed the ability of TRAIL to induce apoptosis in animal models without toxicity toward normal tissues. Therefore, TRAIL is considered to be a tumor-selective, apoptosis-inducing cytokine and a promising new candidate for cancer prevention and treatment. TRAIL binds to several distinct receptors: (a) TRAIL-R1/DR4 (121) (b) TRAIL-R2/DR5 (122) (c) TRAIL-R3/DcR1 (123) and (d) TRAIL-R4/DcR2 (124).

Both DR4 and DR5 contain the intracellular death domain (DD) essential for the induction of apoptosis following receptor ligation (125). In contrast, neither DcR1 nor DcR2 mediates apoptosis due to a complete or partial lack of the intracellular DD, respectively (126, 127). The binding of TRAIL to DR4 and DR5 leads to the activation of caspase-8 or caspase-10 (120, 128, 129), that in turn activates downstream effector caspases such as caspase-3, and caspase-7 (130). Activation of caspase-8 or caspase-10 by TRAIL also cleaves BID (a Bcl-2 inhibitory protein) (131). Truncated BID (tBID) triggers mitochondrial depolarization (decrease in $\Delta\Psi_m$) and causes subsequent release of mitochondrial proteins (cytochrome c, Omi/HtrA2, AIF, and Smac/DIABLO) to cytosol (120, 132-134). Bcl-2/Bcl-X_L preserves mitochondrial transmembrane potential and blocks the release of mitochondrial proteins (12, 13, 22). Once cytochrome c is released into the cytosol, it binds to APAF-1 and, in the presence of dATP, recruits and activates procaspase-9 to form the apoptosome (135). Activated caspases cleave several downstream substrates resulting in activation of endonucleases that ultimately leads to apoptosis (118, 135).

Recently, some TRAIL resistant cancer cell lines have been discovered, but the molecular mechanisms responsible for the TRAIL resistance is still not very clear. We and others have shown that resistance of many types of cancer cells to TRAIL can be reversed by treatment with RNA synthesis inhibitors (84, 128), protein synthesis inhibitors (81, 84, 128), chemotherapeutic agents (136-139) or ionizing radiation (139). Furthermore, we have recently demonstrated that downregulation of Akt/PKB or NF κ B sensitizes breast, prostate and lung cancer cells to TRAIL *in vitro* (81, 84, 140). These studies suggest that combined chemotherapy or radiotherapy with TRAIL could be an effective treatment of epithelial cell-derived cancers. Similarly, we have recently shown that resveratrol enhances the apoptosis-inducing potential of TRAIL in androgen independent PC-3 cells, and sensitizes TRAIL-resistant androgen-dependent prostate cancer LNCaP cells (26, 27). Study of the intracellular mechanisms that control TRAIL sensitivity may enhance our knowledge of death receptor-mediated signaling and help to develop resveratrol and/or TRAIL-based approaches to cancer prevention/treatment.

4. BIOAVAILABILITY AND METABOLISM OF RESVERATROL

Drug metabolism is divided into two phases that involve different enzyme classes. In general, Phase I enzymes, consisting primarily of cytochrome P450s (CYPs) and flavin monooxygenases. These enzymes oxidize, reduce or hydrolyze foreign molecules to convert them in more polar so that their facilitate excretion is possible from the body. Phase II enzymes include conjugating and antioxidant enzymes that are induced in a coordinated manner to detoxify harmful molecules, including toxic products/byproducts of Phase I enzymes. In humans resveratrol rapidly undergoes phase II conjugation, both glucuronidation and sulfation at multiple sites on the molecule. Many other known chemopreventive agents

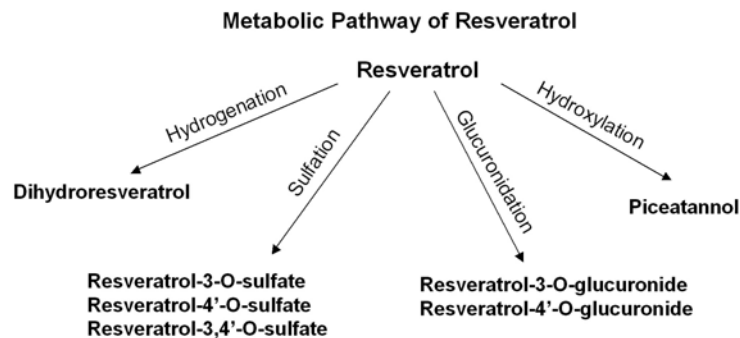


Figure 5. Metabolisms of resveratrol.

upregulate Phase II enzymes, and induction of this pathway is considered to be a promising strategy for cancer prevention in future.

From animal studies and from limited human studies, it appears that resveratrol is absorbed from the gastrointestinal tract following its ingestion. However, the efficiency of its absorption, as well as its distribution, metabolism and excretion (ADME), are not still well understood. It is found in particularly in high concentrations (2 to 40 μM) in red wine (141). As it has been observed that it has a very short half life resulting in low bioavailability.

The metabolism of resveratrol is complex, involving several pathways with significant phase II conjugation to glucuronides and sulfates (Figure 5). Both *cis*- and *trans*-isomers of resveratrol undergo glucuronide conjugation by uridine diphosphate glucuronosyltransferases (UGTs) to form two corresponding glucuronides (3-O-glucuronide and 4'-O-glucuronide) (142, 143). Resveratrol also goes sulfate conjugation by sulfotransferases (SULTs) to form resveratrol 3-O-sulfate in humans (144-146). In addition, several other sulfate conjugates (4'-sulfate, 3,5-disulfate, 3,4'-disulfate, 3,4',5-trisulfate) have been identified in rats (147, 148). The bioformation of resveratrol occurs in two steps, the first being glucuronidation and the second sulfation at higher doses (147, 148). *In vitro* studies have shown that resveratrol can be hydroxylated by CYP1B1 to piceatannol (3,3',4',5-tetrahydroxystilbene), which by itself has anticancer properties and tyrosine kinase inhibitory potential (149). Recent studies have demonstrated the involvement of CYP1A2 in the formation of piceatannol (150). Piceatannol is formed at higher rates in human liver than in intestinal samples. However, recent clinical studies have not demonstrated the generation of piceatannol from resveratrol (151).

Preclinical studies have shown no correlation between the bioavailability of free resveratrol and its chemopreventive or chemotherapeutic efficacy. IC_{50} values for inhibition of cell growth by resveratrol have been reported to be in the range of 5 to 10 μM in preclinical models (152). Based on studies in humans, the bioavailability of free resveratrol appears to be very low. A recent clinical study in humans ($n = 6$) reported plasma concentrations of free resveratrol of approximately 21 nM

after an oral dose of 25 mg. Similar results were reported in three human subjects who received 360 $\mu\text{g/kg}$ resveratrol (153). Studies in animal models (mice, rats, and dogs) and humans have shown that resveratrol undergoes rapid metabolism in the gut to glucuronide and sulfate conjugates, which are well absorbed and result in high circulating levels (about 2 μM) (151, 154). These data indicate that the low bioavailability of free resveratrol will be insufficient to generate the high concentrations ($\geq 5 \mu\text{M}$) that have been demonstrated to be effective in preclinical models. Glucuronidation and sulfation of resveratrol may be important determinants of its pharmacological activity because resveratrol's glucuronide or sulfate conjugates may be deconjugated at the target sites of action, thereby releasing the substrate to elicit biological activity (146, 155, 156). Furthermore, a biologically active resveratrol sulfate or glucuronide can not be ruled out. Although the bioavailability of resveratrol itself is low in humans, peak plasma levels of total resveratrol (combined with glucuronides and sulfates) of up to 2 μM have been found in humans. Hence, the extensive phase II metabolism of resveratrol to its glucuronide or sulfate conjugates may partly explain the disparity between the low circulating levels of resveratrol and the proposed therapeutic efficacy of resveratrol. Resveratrol glucuronidation and sulfation are mediated by specific UGT and SULT isoforms (142, 143, 157), and these enzymes exist in polymorphic states with interethnic variability. Clinical trials with large sample sizes are needed to elucidate the kinetics of resveratrol.

5. CLINICAL SIGNIFICANCE OF RESVERATRO

5.1. Effects of Resveratrol on Cancer

The most advanced stage of a cancer is its ability to spread, or metastasize. Cancer cells initially group together to form a primary tumor. Once the tumor is formed, cells may start to break off from this primary location and travel to other parts of the body through blood or lymphatic system. This process is metastasis. These cancer cells that travel through the body are capable of establishing new tumors in locations far from the site of the original disease.

Administration of the diphenol resveratrol had a clear anti-metastatic effect, decreasing both the number and the weight of the lung metastases. Similar effects were

observed with resveratrol, resulting in an approximately 40% reduction in the number of metastases (158). In a study with resveratrol, it has been found that resveratrol, at doses of 2.5 and 10 mg/kg, significantly reduced the tumor volume (42%), tumor weight (44%) and metastasis to the lung (56%) in mice bearing highly metastatic Lewis lung carcinoma (LLC) tumors (159).

In other studies a inhibition effect of resveratrol on carcinogenesis at multiple stages particularly on skin has been reported (11). In their finding, they concluded that by its topical application the number of skin tumours per mouse reduced by up to 98% gives worldwide attraction among researchers.

5.2. Effects of Resveratrol on Angiogenesis

Angiogenesis is the process of formation of new blood capillaries required to support the growth of most solid tumours. In various studies (159, 160), it has been observed that resveratrol inhibits tumour-induced formation of new blood capillaries. Moreover, its effects on inhibition of vascularization in the corneal micropocket assay in mice has also been found (161). It has been observed that both cyclooxygenase and ODC have a significant role in promotion of angiogenesis, and their suppression by resveratrol suggests its role in its inhibitory effects on vascularization and tumour growth.

5.3. Effects of Resveratrol on Cardiovascular Disease

Several studies suggest that resveratrol is an effective antioxidant (162-165). It inhibits lipid peroxidation of low-density lipoprotein (LDL), prevents the cytotoxicity of oxidized LDL, and protects cells against lipid peroxidation. It is thought that because it contains highly hydrophilic and lipophilic properties, it can provide more effective protection than other well known antioxidants, such as Vitamin C and E (162). More or insufficient aggregation/clumping of platelets can lead to thrombus formation and subsequent blockages in blood vessels that may result in transient ischaemia, myocardial infarction or stroke. Reduced platelet aggregation has also been demonstrated in studies on Resveratrol, further contributing to its prevention of atherosclerosis. The cardioprotective effects of Resveratrol may also be due, in part, to its vasorelaxation properties. The vasorelaxant activity of Resveratrol has been seen due to its ability to stimulate Ca^{2+} -activated K^+ channels and to enhance NO signaling in the endothelium (166, 167).

5.4. Antiviral Effects of Resveratrol

In several studies, resveratrol was found to increase the potency of some antiretroviral drugs synergistically against HIV and herpes simplex viruses, as resveratrol down regulates the expression of NF κ B and thereby suppresses the activation of this transcription- and apoptosis-related protein (168-174). The study further found that multiple viral protein products were reduced or completely blocked, as well as a reduction in viral DNA production (171). It was also found to be effective for treatment for influenza virus by restricting its replication property. In another study, the replication of severe acute respiratory syndrome (SARS) was totally inhibited by resveratrol derivatives *in vitro* (174).

5.5. Effects of Resveratrol on Inflammation

Inflammation processes are mediated by prostaglandins (PGs). Inhibition of PG activity may be partially responsible for the chemopreventive and cardioprotective effects of resveratrol. Resveratrol decreased the expression of cyclooxygenase-2 (COX-2), an enzyme that catalyzes PG synthesis by inhibiting its expression via signal transduction pathways (175). Nuclear factor- κ B, a transcription factor, regulates genes involved in inflammation and tumorigenesis (176). Inhibition of NF κ B activity is a possible mechanism by which resveratrol exerts its anti-inflammatory activity. Inhibition of TNF-induced NF κ B activation by resveratrol has been observed in several cell lines (177).

5.6. Effects of Resveratrol on Diabetes

In one study, resveratrol has a significant effect on plasma glucose level in normal and diabetic rats including the animal model of streptozotocin (STZ)-induced and nicotinamide-STZ-induced (NA-STZ), and insulin-resistant diabetic rats (178). It was observed that resveratrol produced a hypoglycemic effect in a dose-dependent manner in normal as well as in diabetic rats, and it was also found that in both cases the insulin level was increased following resveratrol treatment. The results indicated that the mechanisms contributing to the hypoglycemic effect of resveratrol include insulin-dependent and insulin-independent pathway, along with PI3K-Akt-signaling pathways to enhance glucose uptake in skeletal muscle. In another study, treatment with resveratrol caused a reduction in the levels of malondialdehyde (MDA), xanthine oxidase (XO) and nitric oxide (NO) in the hippocampus, cortex, cerebellum, brain stem and spinal cord, while simultaneous caused an increase in glutathione levels when compared to the streptozotocin-induced diabetic-untreated group (179). This study demonstrates that resveratrol is a potent neuroprotective agent against diabetic oxidative damage. Resveratrol has been suggested in treatment of diabetic neuropathy and its protective effect may be mediated through reduction in oxidative stress and DNA fragmentation (180-182).

5.7. Other Effects of Resveratrol

In addition to other properties, resveratrol has been observed to have its analgesics, protection against hearing loss and enhance lipopolysaccharide-induced anorexia in rats, although it has no anorexic effect when given alone (183-185).

In some studies it has been shown that resveratrol prolongs the lifespan of the *C.elegans* and *D. melanogaster*. In other study with resveratrol, it is found that it also extended the maximum lifespan of a short-lived fish, *Nothobranchius furzeri*, by 59%, and extended its median lifespan by 56%. An apparent increase in swimming performance, an increase in cognitive performance, and a lack of neurofibrillary degeneration has also been noticed. So it has been clearly observed that resveratrol supplementation with food extends vertebrate

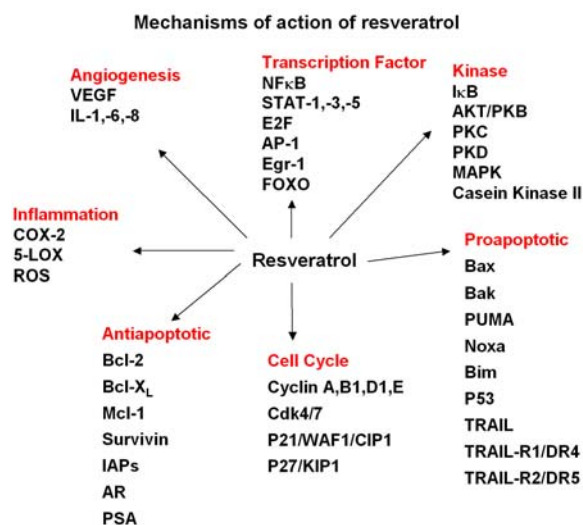


Figure 6. Mechanisms of action of resveratrol.

lifespan and delays motor and cognitive age-related decline could be of high relevance for the prevention of aging-related diseases in the human population (186). The mechanisms of resveratrol's apparent effects on anti-aging, is may be due to its ability to mimic several of the biochemical effects of calorie restriction that may results in lipase inhibition, leading to ultimate reduction in absorption of fat.

6. COMBINATION OF RESVERATROL WITH CHEMOTHERAPY

Resveratrol has been shown to exert sensitization effects on cancer cells that will result in a synergistic cytotoxic activity when resveratrol is used in combination with cytotoxic drugs in drug-resistant tumor cells (187-190). In lung cancer cell lines, it inhibited the growth of A549, EBC-1 and Lu65 lung cancer cells by 50%. Although simultaneous exposure to resveratrol and paclitaxel did not result in significant combined effect, though resveratrol significantly enhanced the subsequent antiproliferative effect of paclitaxel (187). However studies regarding combined effect of resveratrol with quercetin resulted in a gradual and significant increase in the inhibitory effect of quercetin on cell growth and DNA synthesis (169). So it be concluded that resveratrol alone or in combination with quercetin, is an effective inhibitor of oral squamous carcinoma cell (SCC-25) growth and proliferation (169). Resveratrol enhances the growth inhibitory/anticancer activity of cisplatin and doxorubicin *in vitro* and protects against doxorubicin-induced cardiac toxicity both *in vitro* and in mice (189). In colon cancer cells, resveratrol has a direct, dose dependent, inhibitory effect on cell proliferation, and at relatively high concentrations it was found to substantially down-regulate telomerase activity (191). Chemotherapy based on resveratrol and propolis, alone or in combination with vinorelbine has been suggested for prostate cancer therapy (192). Clearly, the studies with resveratrol provide support for its use in human cancer chemoprevention and

combination with chemotherapeutic drugs or cytotoxic factors in the treatment of drug or TRAIL refractory tumors, and provide lead for further investigation as cancer chemopreventive agents.

7. COMBINATION OF RESVERATROL WITH RADIOTHERAPY

Ionizing radiation is widely used in radiotherapy, in order to promote an apoptotic response in cancerous cells. Irradiation alone induced a dose-related reduction in cell proliferation and the appearance of polyploid cells in human leukemia cell line EOL-1 cells (192). Simultaneous exposure to X-irradiation and resveratrol resulted in a synergistic decrease of cell proliferation as well as in a synergistic increase of apoptosis and necrosis. These results suggest that, resveratrol enhance radiation-induced apoptosis in the leukemic cell line, EOL-1. In another study, resveratrol was protective against ultraviolet A (UVA) irradiation-induced cellular damage (193). It could enhance the proliferation activity, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in cancer cells under UVA irradiation, and decrease the content of maleic dialdehyde (MDA).

Ex vivo purging of contaminating tumor cells may reduce the incidence of relapse in patients undergoing bone marrow transplantation. In this study we demonstrate that resveratrol, a phytoalexin with anti-oxidant and chemopreventive activity, exhibits anti-leukemic activity against mouse (32Dp210, L1210) and human (U937, HL-60) leukemic cell lines by inhibiting cell proliferation. Long-term exposure to resveratrol also inhibits the clonal growth of normal hematopoietic progenitor cells but at a higher IC₅₀ of resveratrol than that for most of the leukemia cell lines tested. The inhibitory effect of resveratrol on hematopoietic progenitors is partially reversible, whereas the effect on leukemia cells is largely irreversible. The inhibition of leukemia cells by resveratrol involves nucleosomal DNA fragmentation (apoptosis). On the other hand, resveratrol does not induce or enhance spontaneously occurring apoptotic death in normal hematopoietic progenitor cells. *In vivo* experiments performed with untreated and resveratrol-treated bone marrow showed comparable hematopoietic reconstitution in lethally irradiated mice (10 Gy) as determined by survival, hematologic recovery, and the number of hematopoietic progenitor cells present in the marrow of reconstituted animals. Taken together, these results indicate the potential use of resveratrol for *ex vivo* pharmacological purging of leukemia cells from bone marrow autografts without significant loss in the hematopoietic activity of progenitor cells (194). Prostate cancer DU145 cells are resistant to ionizing radiation-induced apoptosis, but pretreatment with resveratrol significantly enhances apoptosis (195). Resveratrol acts synergistically with ionizing radiation to inhibit cell survival *in vitro*. Resveratrol also potentiates ionizing radiation-induced ceramide accumulation, by promoting its *de novo* biosynthesis, suggesting a critical role of ceramide in resveratrol-induced apoptosis.

8. CONCLUSIONS AND FUTURE DIRECTIONS

Resveratrol is a naturally occurring phytoalexins and exists in *cis*- and *trans*- forms, with *trans*- form being more biologically active. It regulates proliferation, cell cycle, apoptosis, angiogenesis and metastasis through regulation of multiple signaling pathways (Figure 6). Resveratrol inhibits growth of several cancer cell lines and tumors, indicating its anticancerous property. Moreover its role in induction of phase II metabolizing enzymes suggest its detoxifying activity along with upregulation of several apoptotic factors by downregulating transcription factor NF κ B activity thereby affecting the proliferation of cancer cells. These effects may be due to the resemblance of structure of resveratrol with like that of endogenous cell signaling molecules. As by its structure it can be seen that it mimics the molecules that stimulate the oestrogen receptors. Resveratrol also potentiates the apoptotic effects of cytokines, chemotherapeutic agents and gamma-radiation. Pharmacokinetic and pharmacodynamic studies demonstrated that the main target organs of resveratrol are liver and kidney, where it is concentrated after absorption and is metabolized by hydroxylation, glucuronidation, sulfation and hydrogenation. Based on present knowledge, resveratrol appears to be a promising bioactive natural molecule with potential applications in phytotherapy, pharmacology or in nutriprotection area, and could be given as a preventive agent in cancer patients undergoing radiotherapy or chemotherapy. It has been found to be protective from therapy-associated toxicities and there is no doubt that in future it will prove as a most valuable drug of next decade. Furthermore, the ability of resveratrol to protect normal cells and sensitize tumor cells offer additional advantage for the treatment and prevention of human cancer.

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