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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.

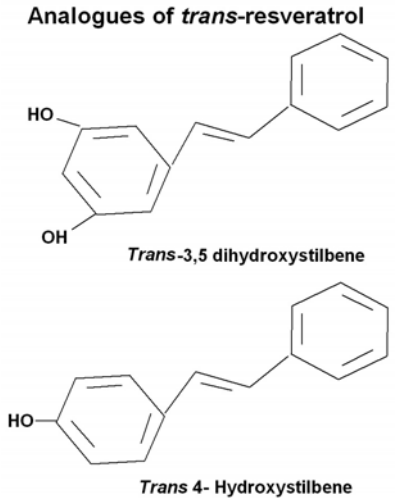

Figure 1. Analogues of *trans*-resveratrol.

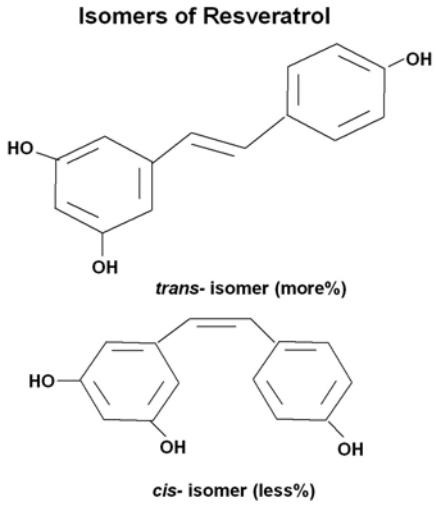

Figure 2. Structure of resveratrol both *cis*- and *trans*-isomers.

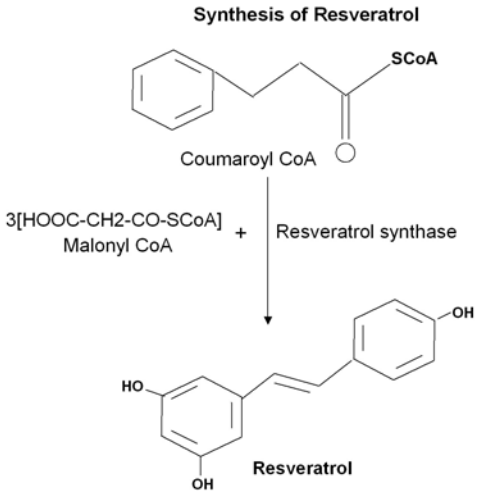

Figure 3. Synthesis of resveratrol.

2. INTRODUCTION

Resveratrol ($C_{14}H_{12}O_3$) 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, find 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).

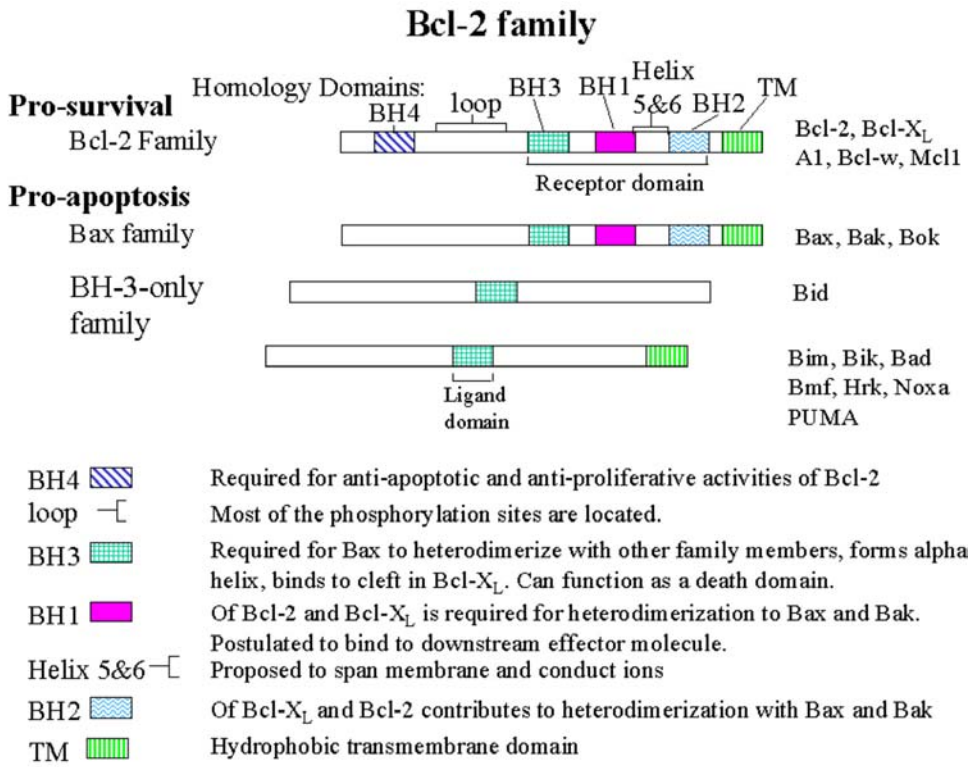

Figure 4. Structure of Bcl-2 family members. Bcl-2 family contains both anti-apoptotic (Bcl-2, Bcl-X_L, A1, Bcl-w and Mcl-1) and pro-apoptotic (Bax, Bak, Bok, Bid, Bim, Bik, Bad, Bmf, Hrk, Noxa and PUMA) proteins. Known α-helical regions are indicated. BH (BH1-4) domains are highly conserved among family 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, G_2 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 G_1 could also imply the Rb or the p53 pathways. Indeed, it was shown that resveratrol treatment of melanoma cells resulted in reduced hyperphosphorylated 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 G_1/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 G_2 phases of the cell-cycle and apoptosis The effect of resveratrol on the G_2-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-κB 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 **w**as 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 NFκB-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 G1, 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 NFκB, 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)/ß-catenin (113), and CREB/CREM proteins (115). However, the constitutive activation of NFκB (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 NFκB 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 NFκB 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 covert 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

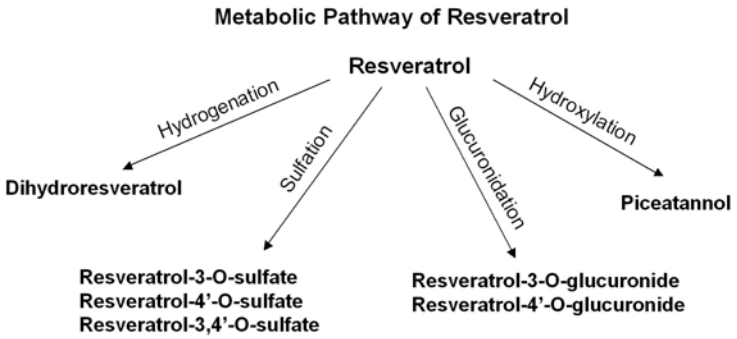

Figure 5. Metabolisms of resveratrol.

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 glucoronosyltransferases (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 μg/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 (≥5 μM) 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 worlwide 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 vasorelaxtant 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 NFκB and there by 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-κB, a transcription factor, regulates genes involved in inflammation and tumirogenesis (176). Inhibition of NFκB activity is a possible mechanism by which resveratrol exerts its anti-inflammatory activity. Inhibition of TNF-induced NFκB 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

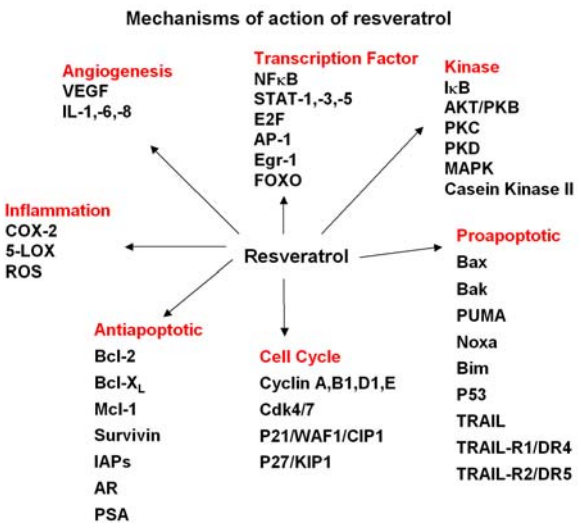

Figure 6. Mechanisms of action of resveratrol.

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 NFκB 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. Phamacokinetic 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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10. REFERENCES

1. Boyle, P., P. Maisonneuve & P. Napalkov: Geographical and temporal patterns of incidence and mortality from prostate cancer. *Urology*, 46, 47-55 (1995)
2. Hong, J. H., K. S. Ahn, E. Bae, S. S. Jeon & H. Y. Choi: The effects of curcumin on the invasiveness of prostate cancer in vitro and in vivo. *Prostate Cancer Prostatic Dis* (2006)
3. Manson, M. M., P. B. Farmer, A. Gescher & W. P. Steward: Innovative agents in cancer prevention. *Recent Results Cancer Res*, 166, 257-75 (2005)
4. Jang, D. S., B. S. Kang, S. Y. Ryu, I. M. Chang, K. R. Min & Y. Kim: Inhibitory effects of resveratrol analogs on unopsonized zymosan-induced oxygen radical production. *Biochem Pharmacol*, 57, 705-12 (1999)
5. Inoue, H., X. F. Jiang, T. Katayama, S. Osada, K. Umesono & S. Namura: Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator-activated receptor alpha in mice. *Neurosci Lett*, 352, 203-6 (2003)
6. Cos, P., T. De Bruyne, S. Apers, D. Vanden Berghe, L. Pieters & A. J. Vlietinck: Phytoestrogens: recent developments. *Planta Med*, 69, 589-99 (2003)
7. Agarwal, R.: Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. *Biochem Pharmacol*, 60, 1051-9 (2000)
8. Mitchell, K. O. & W. S. El-Deiry: Overexpression of c-Myc inhibits p21WAF1/CIP1 expression and induces S-phase entry in 12-O-tetradecanoylphorbol-13-acetate (TPA)-sensitive human cancer cells. *Cell Growth Differ*, 10, 223-30 (1999)
9. Hsieh, T. C. & J. M. Wu: Grape-derived chemopreventive agent resveratrol decreases prostate-specific antigen (PSA) expression in LNCaP cells by an androgen receptor (AR)-independent mechanism. *Anticancer Res*, 20, 225-8 (2000)
10. Bhat, K. P. & J. M. Pezzuto: Cancer chemopreventive activity of resveratrol. *Ann N Y Acad Sci*, 957, 210-29 (2002)
11. Jang, M., L. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. Beecher, H. H. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon & J. M. Pezzuto: Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, 275, 218-20 (1997)
12. Srivastava, R. K., S. J. Sollott, L. Khan, R. Hansford, E. G. Lakatta & D. L. Longo: Bcl-2 and Bcl-X (L) block thapsigargin-induced nitric oxide generation, c-Jun NH (2)-terminal kinase activity, and apoptosis. *Mol Cell Biol*, 19, 5659-74 (1999)
13. Kroemer, G. & J. C. Reed: Mitochondrial control of cell death. *Nat Med*, 6, 513-9 (2000)
14. Korsmeyer, S. J.: BCL-2 gene family and the regulation of programmed cell death. *Cancer Res*, 59, 1693s-1700s (1999)
15. Cory, S., D. C. Huang & J. M. Adams: The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene*, 22, 8590-607 (2003)
16. Oltvai, Z. N., C. L. Milliman & S. J. Korsmeyer: Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell*, 74, 609-19 (1993)
17. Adams, J. M. & S. Cory: The Bcl-2 protein family: arbiters of cell survival. *Science*, 281, 1322-6 (1998)
18. Kelekar, A. & C. B. Thompson: Bcl-2-family proteins: the role of the BH3 domain in apoptosis. *Trends Cell Biol*, 8, 324-30 (1998)
19. Reed, J. C.: Bcl-2 family proteins. *Oncogene*, 17, 3225-36 (1998)
20. McDonnell, J. M., D. Fushman, C. L. Milliman, S. J. Korsmeyer & D. Cowburn: Solution structure of the proapoptotic molecule BID: a structural basis for apoptotic agonists and antagonists. *Cell*, 96, 625-34 (1999)
21. Srivastava, R. K., Q. S. Mi, J. M. Hardwick & D. L. Longo: Deletion of the loop region of Bcl-2 completely blocks paclitaxel-induced apoptosis. *Proc Natl Acad Sci U S A*, 96, 3775-80 (1999)
22. Srivastava, R. K., C. Y. Sasaki, J. M. Hardwick & D. L. Longo: Bcl-2-mediated drug resistance: inhibition of apoptosis by blocking nuclear factor of activated T lymphocytes (NFAT)-induced Fas ligand transcription. *J Exp Med*, 190, 253-65 (1999)

23. Hickman, J. A., C. S. Potten, A. J. Merritt & T. C. Fisher: Apoptosis and cancer chemotherapy. *Philos Trans R Soc Lond B Biol Sci*, 345, 319-25 (1994)
24. Levine, B., J. E. Goldman, H. H. Jiang, D. E. Griffin & J. M. Hardwick: Bc1-2 protects mice against fatal alphavirus encephalitis. *Proc Natl Acad Sci U S A*, 93, 4810-5 (1996)
25. Fulda, S. & K. M. Debatin: Resveratrol-mediated sensitisation to TRAIL-induced apoptosis depends on death receptor and mitochondrial signalling. *Eur J Cancer*, 41, 786-98 (2005)
26. Shankar, S., I. Siddiqui & R. K. Srivastava: Resveratrol sensitizes TRAIL-resistant human prostate cancer cells by generating reactive oxygen species, targeting p53 to mitochondria, and regulating Bcl-2 family members, IAPs and death receptors TRAIL-R1/DR4 and TRAIL-R2/DR5. *Cancer Res*, in press, (2007)
27. Shankar, S., I. Siddiqui & R. K. Srivastava: Molecular mechanisms of resveratrol (3,4,5-trihydroxy-trans-stilbene) and its interaction with TNF-related apoptosis inducing ligand (TRAIL) in androgen-insensitive prostate cancer cells. *Mol Cell Biochem*, in press, (2007)
28. Aggarwal, B. B. & S. Shishodia: Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol*, 71, 1397-421 (2006)
29. Bhardwaj, A., G. Sethi, S. Vadhan-Raj, C. Bueso-Ramos, Y. Takada, U. Gaur, A. S. Nair, S. Shishodia & B. B. Aggarwal: Resveratrol inhibits proliferation, induces apoptosis, and overcomes chemoresistance through down-regulation of STAT3 and nuclear factor-{kappa}B-regulated antiapoptotic and cell survival gene products in human multiple myeloma cells. *Blood*, 109, 2293-302 (2007)
30. Benitez, D. A., E. Pozo-Guisado, A. Alvarez-Barrientos, P. M. Fernandez-Salguero & E. A. Castellon: Mechanisms involved in resveratrol-induced apoptosis and cell cycle arrest in prostate cancer-derived cell lines. *J Androl*, 28, 282-93 (2007)
31. Kotha, A., M. Sekharam, L. Cilenti, K. Siddiquee, A. Khaled, A. S. Zervos, B. Carter, J. Turkson & R. Jove: Resveratrol inhibits Src and Stat3 signaling and induces the apoptosis of malignant cells containing activated Stat3 protein. *Mol Cancer Ther*, 5, 621-9 (2006)
32. Liang, Y. C., S. H. Tsai, L. Chen, S. Y. Lin-Shiau & J. K. Lin: Resveratrol-induced G2 arrest through the inhibition of CDK7 and p34CDC2 kinases in colon carcinoma HT29 cells. *Biochem Pharmacol*, 65, 1053-60 (2003)
33. Ferrer, P., M. Asensi, R. Segarra, A. Ortega, M. Benlloch, E. Obrador, M. T. Varea, G. Asensio, L. Jorda & J. M. Estrela: Association between pterostilbene and quercetin inhibits metastatic activity of B16 melanoma. *Neoplasia*, 7, 37-47 (2005)
34. Maulik, N.: Reactive oxygen species drives myocardial angiogenesis? *Antioxid Redox Signal*, 8, 2161-8 (2006)
35. Zhang, W., Z. Fei, H. N. Zhen, J. N. Zhang & X. Zhang: Resveratrol inhibits cell growth and induces apoptosis of rat C6 glioma cells. *J Neurooncol*, 81, 231-40 (2007)
36. Bruder, J. L., T. Hsieh, K. M. Lerea, S. C. Olson & J. M. Wu: Induced cytoskeletal changes in bovine pulmonary artery endothelial cells by resveratrol and the accompanying modified responses to arterial shear stress. *BMC Cell Biol*, 2, 1 (2001)
37. She, Q. B., A. M. Bode, W. Y. Ma, N. Y. Chen & Z. Dong: Resveratrol-induced activation of p53 and apoptosis is mediated by extracellular-signal-regulated protein kinases and p38 kinase. *Cancer Res*, 61, 1604-10 (2001)
38. Lin, H. Y., A. Shih, F. B. Davis, H. Y. Tang, L. J. Martino, J. A. Bennett & P. J. Davis: Resveratrol induced serine phosphorylation of p53 causes apoptosis in a mutant p53 prostate cancer cell line. *J Urol*, 168, 748-55 (2002)
39. Huang, C., W. Y. Ma, A. Goranson & Z. Dong: Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. *Carcinogenesis*, 20, 237-42 (1999)
40. Kannan, K. & S. K. Jain: Oxidative stress and apoptosis. *Pathophysiology*, 7, 153-163 (2000)
41. Kroemer, G., N. Zamzami & S. A. Susin: Mitochondrial control of apoptosis. *Immunol Today*, 18, 44-51 (1997)
42. Macip, S., M. Igarashi, P. Berggren, J. Yu, S. W. Lee & S. A. Aaronson: Influence of induced reactive oxygen species in p53-mediated cell fate decisions. *Mol Cell Biol*, 23, 8576-85 (2003)
43. Polyak, K., Y. Xia, J. L. Zweier, K. W. Kinzler & B. Vogelstein: A model for p53-induced apoptosis. *Nature*, 389, 300-5 (1997)
44. Chandel, N. S., M. G. Vander Heiden, C. B. Thompson & P. T. Schumacker: Redox regulation of p53 during hypoxia. *Oncogene*, 19, 3840-8 (2000)
45. Bode, A. M. & Z. Dong: Post-translational modification of p53 in tumorigenesis. *Nat Rev Cancer*, 4, 793-805 (2004)
46. Notas, G., A. P. Nifli, M. Kampa, J. Vercauteren, E. Kouroumalis & E. Castanas: Resveratrol exerts its antiproliferative effect on HepG2 hepatocellular carcinoma cells, by inducing cell cycle arrest, and NOS activation. *Biochim Biophys Acta*, 1760, 1657-66 (2006)
47. Chander, V. & K. Chopra: Protective effect of resveratrol, a polyphenolic phytoalexin on glycerol-induced acute renal failure in rat kidney. *Ren Fail*, 28, 161-9 (2006)
48. Holian, O., S. Wahid, M. J. Atten & B. M. Attar: Inhibition of gastric cancer cell proliferation by resveratrol: role of nitric oxide. *Am J Physiol Gastrointest Liver Physiol*, 282, G809-16 (2002)
49. Kim, A. L., Y. Zhu, H. Zhu, L. Han, L. Kopelovich, D. R. Bickers & M. Athar: Resveratrol inhibits proliferation of human epidermoid carcinoma A431 cells by modulating MEK1 and AP-1 signalling pathways. *Exp Dermatol*, 15, 538-46 (2006)
50. Nam, N. H.: Naturally occurring NF-kappaB inhibitors. *Mini Rev Med Chem*, 6, 945-51 (2006)
51. Das, S., V. K. Alagappan, D. Bagchi, H. S. Sharma, N. Maulik & D. K. Das: Coordinated induction of iNOS-VEGF-KDR-eNOS after resveratrol consumption: a potential mechanism for resveratrol preconditioning of the heart. *Vascul Pharmacol*, 42, 281-9 (2005)
52. Madar, Z., N. Maayan, O. Sarit & A. Eliraz: Antioxidants modulate the nitric oxide system and SOD activity and expression in rat epithelial lung cells. *Asia Pac J Clin Nutr*, 13, S101 (2004)
53. Maehama, T. & J. E. Dixon: PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol*, 9, 125-8 (1999)

54. Cairns, P., K. Okami, S. Halachmi, N. Halachmi, M. Esteller, J. G. Herman, J. Jen, W. B. Isaacs, G. S. Bova & D. Sidransky: Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res*, 57, 4997-5000 (1997)
55. Guldberg, P., P. thor Straten, A. Birck, V. Ahrenkiel, A. F. Kirkin & J. Zeuthen: Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequent event in malignant melanoma. *Cancer Res*, 57, 3660-3 (1997)
56. Kohno, T., M. Takahashi, R. Manda & J. Yokota: Inactivation of the PTEN/MMAC1/TEP1 gene in human lung cancers. *Genes Chromosomes Cancer*, 22, 152-6 (1998)
57. Liu, W., C. D. James, L. Frederick, B. E. Alderete & R. B. Jenkins: PTEN/MMAC1 mutations and EGFR amplification in glioblastomas. *Cancer Res*, 57, 5254-7 (1997)
58. Di Cristofano, A. & P. P. Pandolfi: The multiple roles of PTEN in tumor suppression. *Cell*, 100, 387-90 (2000)
59. Dahia, P. L., R. C. Aguiar, J. Alberta, J. B. Kum, S. Caron, H. Sill, D. J. Marsh, J. Ritz, A. Freedman, C. Stiles & C. Eng: PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanismsin haematological malignancies. *Hum Mol Genet*, 8, 185-93 (1999)
60. Wu, G. S., P. Saftig, C. Peters & W. S. El-Deiry: Potential role for cathepsin D in p53-dependent tumor suppression and chemosensitivity. *Oncogene*, 16, 2177-83 (1998)
61. Datta, S. R., A. Brunet & M. E. Greenberg: Cellular survival: a play in three Akts. *Genes Dev*, 13, 2905-27 (1999)
62. Bellacosa, A., D. de Feo, A. K. Godwin, D. W. Bell, J. Q. Cheng, D. A. Altomare, M. Wan, L. Dubeau, G. Scambia, V. Masciullo & et al.: Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. *Int J Cancer*, 64, 280-5 (1995)
63. Coffer, P. J. & J. R. Woodgett: Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. *Eur J Biochem*, 205, 1217 (1992)
64. Datta, S. R., H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh & M. E. Greenberg: Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*, 91, 231-41 (1997)
65. del Peso, L., M. Gonzalez-Garcia, C. Page, R. Herrera & G. Nunez: Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science*, 278, 687-9 (1997)
66. Cardone, M. H., N. Roy, H. R. Stennicke, G. S. Salvesen, T. F. Franke, E. Stanbridge, S. Frisch & J. C. Reed: Regulation of cell death protease caspase-9 by phosphorylation. *Science*, 282, 1318-21 (1998)
67. Kang, J. J., M. D. Schaber, S. M. Srinivasula, E. S. Alnemri, G. Litwack, D. J. Hall & M. A. Bjornsti: Cascades of mammalian caspase activation in the yeast Saccharomyces cerevisiae. *J Biol Chem*, 274, 3189-98 (1999)
68. Brunet, A., A. Bonni, M. J. Zigmond, M. Z. Lin, P. Juo, L. S. Hu, M. J. Anderson, K. C. Arden, J. Blenis & M. E. Greenberg: Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell*, 96, 857-68 (1999)
69. Kops, G. J. & B. M. Burgering: Forkhead transcription factors: new insights into protein kinase B (c-akt) signaling. *J Mol Med*, 77, 656-65 (1999)
70. Romashkova, J. A. & S. S. Makarov: NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature*, 401, 86-90 (1999)
71. Ozes, O. N., L. D. Mayo, J. A. Gustin, S. R. Pfeffer, L. M. Pfeffer & D. B. Donner: NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature*, 401, 82-5 (1999)
72. Datta, S. R., A. Katsov, L. Hu, A. Petros, S. W. Fesik, M. B. Yaffe & M. E. Greenberg: 14-3-3 proteins and survival kinases cooperate to inactivate BAD by BH3 domain phosphorylation. *Mol Cell*, 6, 41-51 (2000)
73. Datta, S. R., A. M. Ranger, M. Z. Lin, J. F. Sturgill, Y. C. Ma, C. W. Cowan, P. Dikkes, S. J. Korsmeyer & M. E. Greenberg: Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. *Dev Cell*, 3, 631-43 (2002)
74. Hill, B. & N. Kyprianou: Sequencing hormonal ablation and radiotherapy in prostate cancer: a molecular and therapeutic prespective (Review). *Oncol Rep*, 9, 1151-6 (2002)
75. Dudek, H., S. R. Datta, T. F. Franke, M. J. Birnbaum, R. Yao, G. M. Cooper, R. A. Segal, D. R. Kaplan & M. E. Greenberg: Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science*, 275, 661-5 (1997)
76. Kaufmann, S. H. & M. O. Hengartner: Programmed cell death: alive and well in the new millennium. *Trends Cell Biol*, 11, 526-34 (2001)
77. Kennedy, S. G., A. J. Wagner, S. D. Conzen, J. Jordan, A. Bellacosa, P. N. Tsichlis & N. Hay: The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev*, 11, 701-13 (1997)
78. Khwaja, A.: Akt is more than just a Bad kinase. *Nature*, 401, 33-4 (1999)
79. Kulik, G. & M. J. Weber: Akt-dependent and -independent survival signaling pathways utilized by insulin-like growth factor I. *Mol Cell Biol*, 18, 6711-8 (1998)
80. Chen, R. H., Y. H. Su, R. L. Chuang & T. Y. Chang: Suppression of transforming growth factor-beta-induced apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway. *Oncogene*, 17, 1959-68 (1998)
81. Kandasamy, K. & R. K. Srivastava: Role of the phosphatidylinositol 3'-kinase/PTEN/Akt kinase pathway in tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in non-small cell lung cancer cells. *Cancer Res*, 62, 4929-37 (2002)
82. Hill, M. M. & B. A. Hemmings: Inhibition of protein kinase B/Akt. implications for cancer therapy. *Pharmacol Ther*, 93, 243-51 (2002)
83. Graff, J. R., B. W. Konicek, A. M. McNulty, Z. Wang, K. Houck, S. Allen, J. D. Paul, A. Hbaiu, R. G. Goode, G. E. Sandusky, R. L. Vessella & B. L. Neubauer: Increased AKT activity contributes to prostate cancer progression by dramatically accelerating prostate tumor growth and diminishing p27Kip1 expression. *J Biol Chem*, 275, 24500-5 (2000)

84. Chen, Q., B. Gong, A. S. Mahmoud-Ahmed, A. Zhou, E. D. Hsi, M. Hussein & A. Almasan: Apo2L/TRAIL and Bcl-2-related proteins regulate type I interferon-induced apoptosis in multiple myeloma. *Blood*, 98, 2183-92 (2001)
85. Ghosh, S., M. J. May & E. B. Kopp: NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol*, 16, 225-60 (1998)
86. Baldwin, A. S., Jr.: The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol*, 14, 649-83 (1996)
87. Karin, M. & Y. Ben-Neriah: Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol*, 18, 621-63 (2000)
88. Mayo, M. W. & A. S. Baldwin: The transcription factor NF-kappaB: control of oncogenesis and cancer therapy resistance. *Biochim Biophys Acta*, 1470, M55-62 (2000)
89. Gilmore, T. D., M. Koedood, K. A. Piffat & D. W. White: Rel/NF-kappaB/IkappaB proteins and cancer. *Oncogene*, 13, 1367-78 (1996)
90. Luque, I. & C. Gelinas: Rel/NF-kappa B and I kappa B factors in oncogenesis. *Semin Cancer Biol*, 8, 103-11 (1997)
91. Pahl, H. L.: Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*, 18, 6853-66 (1999)
92. Kim, D. W., M. A. Sovak, G. Zanieski, G. Nonet, R. Romieu-Mourez, A. W. Lau, L. J. Hafer, P. Yaswen, M. Stampfer, A. E. Rogers, J. Russo & G. E. Sonenshein: Activation of NF-kappaB/Rel occurs early during neoplastic transformation of mammary cells. *Carcinogenesis*, 21, 871-9 (2000)
93. Pestell, R. G., C. Albanese, A. T. Reutens, J. E. Segall, R. J. Lee & A. Arnold: The cyclins and cyclin-dependent kinase inhibitors in hormonal regulation of proliferation and differentiation. *Endocr Rev*, 20, 501-34 (1999)
94. Joyce, D., C. Albanese, J. Steer, M. Fu, B. Bouzahzah & R. G. Pestell: NF-kappaB and cell-cycle regulation: the cyclin connection. *Cytokine Growth Factor Rev*, 12, 73-90 (2001)
95. Gillett, C., V. Fantl, R. Smith, C. Fisher, J. Bartek, C. Dickson, D. Barnes & G. Peters: Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. *Cancer Res*, 54, 1812-7 (1994)
96. McIntosh, G. G., J. J. Anderson, I. Milton, M. Steward, A. H. Parr, M. D. Thomas, J. A. Henry, B. Angus, T. W. Lennard & C. H. Horne: Determination of the prognostic value of cyclin D1 overexpression in breast cancer. *Oncogene*, 11, 885-91 (1995)
97. Wang, L., T. Habuchi, K. Mitsumori, Z. Li, T. Kamoto, H. Kinoshita, N. Tsuchiya, K. Sato, C. Ohyama, A. Nakamura, O. Ogawa & T. Kato: Increased risk of prostate cancer associated with AA genotype of cyclin D1 gene A870G polymorphism. *Int J Cancer*, 103, 116-20 (2003)
98. Reissmann, P. T., H. Koga, R. A. Figlin, E. C. Holmes & D. J. Slamon: Amplification and overexpression of the cyclin D1 and epidermal growth factor receptor genes in non-small-cell lung cancer. Lung Cancer Study Group. *J Cancer Res Clin Oncol*, 125, 61-70 (1999)
99. Le Marchand, L., A. Seifried, A. Lum-Jones, T. Donlon & L. R. Wilkens: Association of the cyclin D1 A870G polymorphism with advanced colorectal cancer. *Jama*, 290, 2843-8 (2003)
100. Bringuier, P. P., Y. Tamimi, E. Schuuring & J. Schalken: Expression of cyclin D1 and EMS1 in bladder tumours; relationship with chromosome 11q13 amplification. *Oncogene*, 12, 1747-53 (1996)
101. Wang, L., T. Habuchi, T. Takahashi, K. Mitsumori, T. Kamoto, Y. Kakehi, H. Kakinuma, K. Sato, A. Nakamura, O. Ogawa & T. Kato: Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. *Carcinogenesis*, 23, 257-64 (2002)
102. Hung, W. C., C. Y. Chai, J. S. Huang & L. Y. Chuang: Expression of cyclin D1 and c-Ki-ras gene product in human epithelial ovarian tumors. *Hum Pathol*, 27, 1324-8 (1996)
103. Zhang, Y. J., W. Jiang, C. J. Chen, C. S. Lee, S. M. Kahn, R. M. Santella & I. B. Weinstein: Amplification and overexpression of cyclin D1 in human hepatocellular carcinoma. *Biochem Biophys Res Commun*, 196, 1010-6 (1993)
104. Gansauge, S., F. Gansauge, M. Ramadani, H. Stobbe, B. Rau, N. Harada & H. G. Beger: Overexpression of cyclin D1 in human pancreatic carcinoma is associated with poor prognosis. *Cancer Res*, 57, 1634-7 (1997)
105. Cavalla, P., A. Dutto, R. Piva, P. Richiardi, R. Grosso & D. Schiffer: Cyclin D1 expression in gliomas. *Acta Neuropathol (Berl)*, 95, 131-5 (1998)
106. Sauter, E. R., U. C. Yeo, A. von Stemm, W. Zhu, S. Litwin, D. S. Tichansky, G. Pistritto, M. Nesbit, D. Pinkel, M. Herlyn & B. C. Bastian: Cyclin D1 is a candidate oncogene in cutaneous melanoma. *Cancer Res*, 62, 3200-6 (2002)
107. Kim, H., E. K. Ham, Y. I. Kim, J. G. Chi, H. S. Lee, S. H. Park, Y. M. Jung, N. K. Myung, M. J. Lee & J. J. Jang: Overexpression of cyclin D1 and cdk4 in tumorigenesis of sporadic hepatoblastomas. *Cancer Lett*, 131, 177-83 (1998)
108. Hemmer, S., V. M. Wasenius, C. Haglund, Y. Zhu, S. Knuutila, K. Franssila & H. Joensuu: Deletion of 11q23 and cyclin D1 overexpression are frequent aberrations in parathyroid adenomas. *Am J Pathol*, 158, 1355-62 (2001)
109. Bergsagel, P. L. & W. M. Kuehl: Critical roles for immunoglobulin translocations and cyclin D dysregulation in multiple myeloma. *Immunol Rev*, 194, 96-104 (2003)
110. Liu, J. H., C. C. Yen, Y. C. Lin, J. P. Gau, M. H. Yang, T. C. Chao, L. T. Hsiao, W. S. Wang, Y. C. Tsai & P. M. Chen: Overexpression of cyclin D1 in accelerated-phase chronic myeloid leukemia. *Leuk Lymphoma*, 45, 2419-25 (2004)
111. Albanese, C., J. Johnson, G. Watanabe, N. Eklund, D. Vu, A. Arnold & R. G. Pestell: Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions. *J Biol Chem*, 270, 23589-97 (1995)
112. Matsumura, I., T. Kitamura, H. Wakao, H. Tanaka, K. Hashimoto, C. Albanese, J. Downward, R. G. Pestell & Y. Kanakura: Transcriptional regulation of the cyclin D1 promoter by STAT5: its involvement in cytokine-dependent growth of hematopoietic cells. *Embo J*, 18, 1367-77 (1999)
113. Shtutman, M., J. Zhurinsky, I. Simcha, C. Albanese, M. D'Amico, R. Pestell & A. Ben-Ze'ev: The cyclin D1

gene is a target of the beta-catenin/LEF-1 pathway. *Proc Natl Acad Sci U S A*, 96, 5522-7 (1999)
114. Watanabe, G., C. Albanese, R. J. Lee, A. Reutens, G. Vairo, B. Henglein & R. G. Pestell: Inhibition of cyclin D1 kinase activity is associated with E2F-mediated inhibition of cyclin D1 promoter activity through E2F and Sp1. *Mol Cell Biol*, 18, 3212-22 (1998)
115. Watanabe, G., A. Howe, R. J. Lee, C. Albanese, I. W. Shu, A. N. Karnezis, L. Zon, J. Kyriakis, K. Rundell & R. G. Pestell: Induction of cyclin D1 by simian virus 40 small tumor antigen. *Proc Natl Acad Sci U S A*, 93, 12861-6 (1996)
116. Wang, T. C., R. D. Cardiff, L. Zukerberg, E. Lees, A. Arnold & E. V. Schmidt: Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature*, 369, 669-71 (1994)
117. Walczak, H., R. E. Miller, K. Ariail, B. Gliniak, T. S. Griffith, M. Kubin, W. Chin, J. Jones, A. Woodward, T. Le, C. Smith, P. Smolak, R. G. Goodwin, C. T. Rauch, J. C. Schuh & D. H. Lynch: Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat Med*, 5, 157-63 (1999)
118. Srivastava, R. K.: Intracellular mechanisms of TRAIL and its role in cancer therapy. *Mol Cell Biol Res Commun*, 4, 67-75 (2000)
119. Bodmer, J. L., N. Holler, S. Reynard, P. Vinciguerra, P. Schneider, P. Juo, J. Blenis & J. Tschopp: TRAIL receptor-2 signals apoptosis through FADD and caspase-8. *Nat Cell Biol*, 2, 241-3 (2000)
120. Suliman, A., A. Lam, R. Datta & R. K. Srivastava: Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. *Oncogene*, 20, 2122-33 (2001)
121. Pan, G., K. O'Rourke, A. M. Chinnaiyan, R. Gentz, R. Ebner, J. Ni & V. M. Dixit: The receptor for the cytotoxic ligand TRAIL. *Science*, 276, 111-3 (1997)
122. Walczak, H., M. A. Degli-Esposti, R. S. Johnson, P. J. Smolak, J. Y. Waugh, N. Boiani, M. S. Timour, M. J. Gerhart, K. A. Schooley, C. A. Smith, R. G. Goodwin & C. T. Rauch: TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. *Embo J*, 16, 5386-97 (1997)
123. Degli-Esposti, M. A., P. J. Smolak, H. Walczak, J. Waugh, C. P. Huang, R. F. DuBose, R. G. Goodwin & C. A. Smith: Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J Exp Med*, 186, 1165-70 (1997)
124. Degli-Esposti, M. A., W. C. Dougall, P. J. Smolak, J. Y. Waugh, C. A. Smith & R. G. Goodwin: The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. *Immunity*, 7, 813-20 (1997)
125. Golstein, P.: Cell death: TRAIL and its receptors. *Curr Biol*, 7, R750-3 (1997)
126. Sheridan, J. P., S. A. Marsters, R. M. Pitti, A. Gurney, M. Skubatch, D. Baldwin, L. Ramakrishnan, C. L. Gray, K. Baker, W. I. Wood, A. D. Goddard, P. Godowski & A. Ashkenazi: Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science*, 277, 818-21 (1997)
127. Marsters, S. A., J. P. Sheridan, R. M. Pitti, A. Huang, M. Skubatch, D. Baldwin, J. Yuan, A. Gurney, A. D. Goddard, P. Godowski & A. Ashkenazi: A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr Biol*, 7, 1003-6 (1997)
128. Griffith, T. S., W. A. Chin, G. C. Jackson, D. H. Lynch & M. Z. Kubin: Intracellular regulation of TRAIL-induced apoptosis in human melanoma cells. *J Immunol*, 161, 2833-40 (1998)
129. Ashkenazi, A. & V. M. Dixit: Death receptors: signaling and modulation. *Science*, 281, 1305-8 (1998)
130. Muzio, M.: Signalling by proteolysis: death receptors induce apoptosis. *Int J Clin Lab Res*, 28, 141-7 (1998)
131. Wang, C. Y., M. W. Mayo & A. S. Baldwin, Jr.: TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. *Science*, 274, 784-7 (1996)
132. Kandasamy, K., S. M. Srinivasula, E. S. Alnemri, C. B. Thompson, S. J. Korsmeyer, J. L. Bryant & R. K. Srivastava: Involvement of proapoptotic molecules Bax and Bak in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced mitochondrial disruption and apoptosis: differential regulation of cytochrome c and Smac/DIABLO release. *Cancer Res*, 63, 1712-21 (2003)
133. Srinivasula, S. M., S. Gupta, P. Datta, Z. Zhang, R. Hegde, N. Cheong, T. Fernandes-Alnemri & E. S. Alnemri: Inhibitor of apoptosis proteins are substrates for the mitochondrial serine protease Omi/HtrA2. *J Biol Chem*, 278, 31469-72 (2003)
134. Kroemer, G.: Mitochondrial control of apoptosis: an overview. *Biochem Soc Symp*, 66, 1-15 (1999)
135. Green, D. R.: Apoptotic pathways: the roads to ruin. *Cell*, 94, 695-8 (1998)
136. Ashkenazi, A., R. C. Pai, S. Fong, S. Leung, D. A. Lawrence, S. A. Marsters, C. Blackie, L. Chang, A. E. McMurtrey, A. Hebert, L. DeForge, I. L. Koumenis, D. Lewis, L. Harris, J. Bussiere, H. Koeppen, Z. Shahrokh & R. H. Schwall: Safety and antitumor activity of recombinant soluble Apo2 ligand. *J Clin Invest*, 104, 155-62 (1999)
137. Keane, M. M., S. A. Ettenberg, M. M. Nau, E. K. Russell & S. Lipkowitz: Chemotherapy augments TRAIL-induced apoptosis in breast cell lines. *Cancer Res*, 59, 734-41 (1999)
138. Singh, A., P. S. Naidu & S. K. Kulkarni: Reversal of aging and chronic ethanol-induced cognitive dysfunction by quercetin a bioflavonoid. *Free Radic Res*, 37, 1245-52 (2003)
139. Shankar, S., T. R. Singh, X. Chen, H. Thakkar, J. Firnin & R. K. Srivastava: The sequential treatment with ionizing radiation followed by TRAIL/Apo-2L reduces tumor growth and induces apoptosis of breast tumor xenografts in nude mice. *Int J Oncol*, 24, 1133-40 (2004)
140. Chen, Q., S. Ray, M. A. Hussein, G. Srkalovic & A. Almasan: Role of Apo2L/TRAIL and Bcl-2-family proteins in apoptosis of multiple myeloma. *Leuk Lymphoma*, 44, 1209-14 (2003)
141. Kopp, P.: Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'? *Eur J Endocrinol*, 138, 619-20 (1998)
142. Aumont, V., S. Krisa, E. Battaglia, P. Netter, T. Richard, J. M. Merillon, J. Magdalou & N. Sabolovic: Regioselective and stereospecific glucuronidation of trans- and cis-resveratrol in human. *Arch Biochem Biophys*, 393, 281-9 (2001)
143. Brill, S. S., A. M. Furimsky, M. N. Ho, M. J. Furniss, Y. Li, A. G. Green, W. W. Bradford, C. E. Green, I. M.

Kapetanovic & L. V. Iyer: Glucuronidation of trans-resveratrol by human liver and intestinal microsomes and UGT isoforms. *J Pharm Pharmacol*, 58, 469-79 (2006)
144. De Santi, C., A. Pietrabissa, R. Spisni, F. Mosca & G. M. Pacifici: Sulphation of resveratrol, a natural compound present in wine, and its inhibition by natural flavonoids. *Xenobiotica*, 30, 857-66 (2000)
145. De Santi, C., A. Pietrabissa, R. Spisni, F. Mosca & G. M. Pacifici: Sulphation of resveratrol, a natural product present in grapes and wine, in the human liver and duodenum. *Xenobiotica*, 30, 609-17 (2000)
146. Walle, T., F. Hsieh, M. H. DeLegge, J. E. Oatis, Jr. & U. K. Walle: High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos*, 32, 1377-82 (2004)
147. Wenzel, E., T. Soldo, H. Erbersdobler & V. Somoza: Bioactivity and metabolism of trans-resveratrol orally administered to Wistar rats. *Mol Nutr Food Res*, 49, 482-94 (2005)
148. Wenzel, E. & V. Somoza: Metabolism and bioavailability of trans-resveratrol. *Mol Nutr Food Res*, 49, 472-81 (2005)
149. Potter, G. A., L. H. Patterson, E. Wanogho, P. J. Perry, P. C. Butler, T. Ijaz, K. C. Ruparelia, J. H. Lamb, P. B. Farmer, L. A. Stanley & M. D. Burke: The cancer preventative agent resveratrol is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1. *Br J Cancer*, 86, 774-8 (2002)
150. Piver, B., M. Fer, X. Vitrac, J. M. Merillon, Y. Dreano, F. Berthou & D. Lucas: Involvement of cytochrome P450 1A2 in the biotransformation of trans-resveratrol in human liver microsomes. *Biochem Pharmacol*, 68, 773-82 (2004)
151. Walle, T., U. K. Walle, D. Sedmera & M. Klausner: Benzo[A]pyrene-induced oral carcinogenesis and chemoprevention: studies in bioengineered human tissue. *Drug Metab Dispos*, 34, 346-50 (2006)
152. Gescher, A. J. & W. P. Steward: Relationship between mechanisms, bioavailibility, and preclinical chemopreventive efficacy of resveratrol: a conundrum. *Cancer Epidemiol Biomarkers Prev*, 12, 953-7 (2003)
153. Goldberg, D. M., J. Yan & G. J. Soleas: Absorption of three wine-related polyphenols in three different matrices by healthy subjects. *Clin Biochem*, 36, 79-87 (2003)
154. Yu, C., Y. G. Shin, A. Chow, Y. Li, J. W. Kosmeder, Y. S. Lee, W. H. Hirschelman, J. M. Pezzuto, R. G. Mehta & R. B. van Breemen: Human, rat, and mouse metabolism of resveratrol. *Pharm Res*, 19, 1907-14 (2002)
155. Kuhnle, G., J. P. Spencer, G. Chowrimootoo, H. Schroeter, E. S. Debnam, S. K. Srai, C. Rice-Evans & U. Hahn: Resveratrol is absorbed in the small intestine as resveratrol glucuronide. *Biochem Biophys Res Commun*, 272, 212-7 (2000)
156. Signorelli, P. & R. Ghidoni: Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. *J Nutr Biochem*, 16, 449-66 (2005)
157. Miksits, M., A. Maier-Salamon, S. Aust, T. Thalhammer, G. Reznicek, O. Kunert, E. Haslinger, T. Szekeres & W. Jaeger: Sulfation of resveratrol in human liver: evidence of a major role for the sulfotransferases SULT1A1 and SULT1E1. *Xenobiotica*, 35, 1101-19 (2005)
158. Busquets, S., E. Ametller, G. Fuster, M. Olivan, V. Raab, J. M. Argiles & F. J. Lopez-Soriano: Resveratrol, a natural diphenol, reduces metastatic growth in an experimental cancer model. *Cancer Lett*, 245, 144-8 (2007)
159. Kimura, Y. & H. Okuda: Resveratrol isolated from Polygonum cuspidatum root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice. *J Nutr*, 131, 1844-9 (2001)
160. Tseng, S. H., S. M. Lin, J. C. Chen, Y. H. Su, H. Y. Huang, C. K. Chen, P. Y. Lin & Y. Chen: Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. *Clin Cancer Res*, 10, 2190-202 (2004)
161. Brakenhielm, E., R. Cao & Y. Cao: Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. *Faseb J*, 15, 1798-800 (2001)
162. Chanvitayapongs, S., B. Draczynska-Lusiak & A. Y. Sun: Amelioration of oxidative stress by antioxidants and resveratrol in PC12 cells. *Neuroreport*, 8, 1499-502 (1997)
163. Belguendouz, L., L. Fremont & M. T. Gozzelino: Interaction of transresveratrol with plasma lipoproteins. *Biochem Pharmacol*, 55, 811-6 (1998)
164. Rotondo, S., G. Rajtar, S. Manarini, A. Celardo, D. Rotillo, G. de Gaetano, V. Evangelista & C. Cerletti: Effect of trans-resveratrol, a natural polyphenolic compound, on human polymorphonuclear leukocyte function. *Br J Pharmacol*, 123, 1691-9 (1998)
165. Frankel, E. N., A. L. Waterhouse & J. E. Kinsella: Inhibition of human LDL oxidation by resveratrol. *Lancet*, 341, 1103-4 (1993)
166. Li, H. F., S. A. Chen & S. N. Wu: Evidence for the stimulatory effect of resveratrol on Ca (2+)-activated K+ current in vascular endothelial cells. *Cardiovasc Res*, 45, 1035-45 (2000)
167. Orallo, F., E. Alvarez, M. Camina, J. M. Leiro, E. Gomez & P. Fernandez: The possible implication of trans-Resveratrol in the cardioprotective effects of long-term moderate wine consumption. *Mol Pharmacol*, 61, 294-302 (2002)
168. Bhat, K. P. L., J. W. Kosmeder, 2nd & J. M. Pezzuto: Biological effects of resveratrol. *Antioxid Redox Signal*, 3, 1041-64 (2001)
169. ElAttar, T. M. & A. S. Virji: Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation. *Anticancer Drugs*, 10, 187-93 (1999)
170. Heredia, A., C. Davis & R. Redfield: Synergistic inhibition of HIV-1 in activated and resting peripheral blood mononuclear cells, monocyte-derived macrophages, and selected drug-resistant isolates with nucleoside analogues combined with a natural product, resveratrol. *J Acquir Immune Defic Syndr*, 25, 246-55 (2000)
171. Faith, S. A., T. J. Sweet, E. Bailey, T. Booth & J. J. Docherty: Resveratrol suppresses nuclear factor-kappaB in herpes simplex virus infected cells. *Antiviral Res*, 72, 242-51 (2006)
172. Naesens, L., P. Bonnafous, H. Agut & E. De Clercq: Antiviral activity of diverse classes of broad-acting agents and natural compounds in HHV-6-infected lymphoblasts. *J Clin Virol*, 37 Suppl 1, S69-75 (2006)
173. Docherty, J. J., T. J. Sweet, E. Bailey, S. A. Faith & T. Booth: Resveratrol inhibition of varicella-zoster virus replication in vitro. *Antiviral Res*, 72, 171-7 (2006)

174. Li, Y. Q., Z. L. Li, W. J. Zhao, R. X. Wen, Q. W. Meng & Y. Zeng: Synthesis of stilbene derivatives with inhibition of SARS coronavirus replication. *Eur J Med Chem*, 41, 1084-9 (2006)
175. Subbaramaiah, K., W. J. Chung, P. Michaluart, N. Telang, T. Tanabe, H. Inoue, M. Jang, J. M. Pezzuto & A. J. Dannenberg: Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. *J Biol Chem*, 273, 21875-82 (1998)
176. Baeuerle, P. A. & V. R. Baichwal: NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. *Adv Immunol*, 65, 111-37 (1997)
177. Holmes-McNary, M. & A. S. Baldwin, Jr.: Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. *Cancer Res*, 60, 3477-83 (2000)
178. Chi, T. C., W. P. Chen, T. L. Chi, T. F. Kuo, S. S. Lee, J. T. Cheng & M. J. Su: Phosphatidylinositol-3-kinase is involved in the antihyperglycemic effect induced by resveratrol in streptozotocin-induced diabetic rats. *Life Sci* (2007)
179. Ates, O., S. Cayli, E. Altinoz, I. Gurses, N. Yucel, M. Sener, A. Kocak & S. Yologlu: Neuroprotection by resveratrol against traumatic brain injury in rats. *Mol Cell Biochem*, 294, 137-44 (2007)
180. Sharma, S., K. Chopra & S. K. Kulkarni: Effect of insulin and its combination with resveratrol or curcumin in attenuation of diabetic neuropathic pain: participation of nitric oxide and TNF-alpha. *Phytother Res*, 21, 278-83 (2007)
181. Su, H. C., L. M. Hung & J. K. Chen: Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. *Am J Physiol Endocrinol Metab*, 290, E1339-46 (2006)
182. Sharma, S., M. Anjaneyulu, S. K. Kulkarni & K. Chopra: Resveratrol, a polyphenolic phytoalexin, attenuates diabetic nephropathy in rats. *Pharmacology*, 76, 69-75 (2006)
183. Gentilli, M., J. X. Mazoit, H. Bouaziz, D. Fletcher, R. F. Casper, D. Benhamou & J. F. Savouret: Resveratrol decreases hyperalgesia induced by carrageenan in the rat hind paw. *Life Sci*, 68, 1317-21 (2001)
184. Torres-Lopez, J. E., M. I. Ortiz, G. Castaneda-Hernandez, R. Alonso-Lopez, R. Asomoza-Espinosa & V. Granados-Soto: Comparison of the antinociceptive effect of celecoxib, diclofenac and resveratrol in the formalin test. *Life Sci*, 70, 1669-76 (2002)
185. Granados-Soto, V., C. F. Arguelles & M. I. Ortiz: The peripheral antinociceptive effect of resveratrol is associated with activation of potassium channels. *Neuropharmacology*, 43, 917-23 (2002)
186. Valenzano, D. R. & A. Cellerino: Resveratrol and the pharmacology of aging: a new vertebrate model to validate an old molecule. *Cell Cycle*, 5, 1027-32 (2006)
187. Kubota, T., Y. Uemura, M. Kobayashi & H. Taguchi: Combined effects of resveratrol and paclitaxel on lung cancer cells. *Anticancer Res*, 23, 4039-46 (2003)
188. Duraj, J., J. Bodo, M. Sulikova, P. Rauko & J. Sedlak: Diverse resveratrol sensitization to apoptosis induced by anticancer drugs in sensitive and resistant leukemia cells. *Neoplasma*, 53, 384-92 (2006)
189. Rezk, Y. A., S. S. Balulad, R. S. Keller & J. A. Bennett: Use of resveratrol to improve the effectiveness of cisplatin and doxorubicin: study in human gynecologic cancer cell lines and in rodent heart. *Am J Obstet Gynecol*, 194, e23-6 (2006)
190. Fulda, S. & K. M. Debatin: Modulation of TRAIL signaling for cancer therapy. *Vitam Horm*, 67, 275-90 (2004)
191. Fuggetta, M. P., G. Lanzilli, M. Tricarico, A. Cottarelli, R. Falchetti, G. Ravagnan & E. Bonmassar: Effect of resveratrol on proliferation and telomerase activity of human colon cancer cells in vitro. *J Exp Clin Cancer Res*, 25, 189-93 (2006)
192. Scifo, C., A. Milasi, A. Guarnera, F. Sinatra & M. Renis: Resveratrol and propolis extract: an insight into the morphological and molecular changes induced in DU145 cells. *Oncol Res*, 15, 409-21 (2006)
193. Chen, M. L., J. Li, W. R. Xiao, L. Sun, H. Tang, L. Wang, L. Y. Wu, X. Chen & H. F. Xie: Protective effect of resveratrol against oxidative damage of UVA irradiated HaCaT cells. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*, 31, 635-9 (2006)
194. Gautam, S. C., Y. X. Xu, M. Dumaguin, N. Janakiraman & R. A. Chapman: Resveratrol selectively inhibits leukemia cells: a prospective agent for ex vivo bone marrow purging. *Bone Marrow Transplant*, 25, 639-45 (2000)
195. Scarlatti, F., G. Sala, C. Ricci, C. Maioli, F. Milani, M. Minella, M. Botturi & R. Ghidoni: Resveratrol sensitization of DU145 prostate cancer cells to ionizing radiation is associated to ceramide increase. *Cancer Lett* (2007)

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