

Resveratrol and Cancer: Chemoprevention, Apoptosis, and Chemo-immunosensitizing Activities

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Abstract: The polyphenolic compound Resveratrol is a naturally occurring phytochemical and can be found in many plant species, including grapes, peanuts and various herbs. Several studies have established that Resveratrol can exert anti-oxidant and anti-inflammatory activities. It also has activity in the regulation of multiple cellular events associated with carcinogenesis. This review describes the general properties of Resveratrol including its relationship to estrogen, its effect on lipid metabolism, its cardiovascular effects, and its role on gene expression. Resveratrol has also been examined in several model systems for its potential effect against cancer. Its anti-cancer 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. In addition, 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. Clearly, the studies with Resveratrol provide support for the use of Resveratrol in human cancer chemoprevention and combination with chemotherapeutic drugs or cytotoxic factors in the treatment of drug refractory tumor cells.

I. INTRODUCTION

Wine has been part of human culture for 6,000 years, serving dietary and socio-religious functions. Its production takes place in every continent, and its chemical composition is profoundly influenced by ecological techniques, the grape cultivar from which it originates, and factors that affect the climate. In addition to ethanol, which in moderate consumption can reduce mortality from coronary heart disease by increasing high-density lipoprotein cholesterol and inhibiting platelet aggregation, wine (especially red wine) contains a range of polyphenols that have desirable biological properties. These include the phenolic acids (p-coumaric, cinnamic, caffeic, gentisic, ferulic, and vanillic acids), trihydroxy stilbenes (Resveratrol and polydatin), and flavonoids (catechin, epicatechin, and quercetin). They are synthesized by a common pathway from phenylalanine involving polyketide condensation reactions. Several of these compounds (notably catechin, quercetin, and Resveratrol) promote nitric oxide production by vascular endothelium; inhibit the synthesis of thromboxane in platelets and leukotriene in neutrophils, modulate the synthesis and secretion of lipoproteins in whole animals and human cell lines, and arrest tumor growth as well as inhibit carcinogenesis in different experimental models.

Resveratrol (*trans*-3,5,4'-trihydroxystilbene, MW 228.2) (Fig. 1) is the parent compound of a family of molecules

including glucosides and polymers, existing in *cis* and *trans* configurations in narrow range of spermatophytes of which vines, peanuts and pines are the prime representatives [155]. The source of Resveratrol in the human diet is the red wine, and it has been proposed to be a constituent of the polyphenol fraction to which the health benefits of red wine consumption have been attributed.

Until 1992, there was no interest in Resveratrol from the perspective of mammalian biochemistry or clinical science, but in that year, Siemann and Creasy reported the presence of *trans*-Resveratrol in wine [153]. This report drew attention to the fact that Resveratrol was also a constituent of oriental folk medicines that were considered to benefit persons afflicted by a variety of disorders including those affecting the liver, skin, heart and circulation, and the lipid metabolism [123, 94].

Interest of Resveratrol in the medical literature was the result of the identification of Resveratrol in the root of the plant *Polygonum cuspidatum* [22]. This plant preparation is a major ingredient of Ko-jo-kon, a Japanese folk medicine reputed for its therapeutic effects upon humans suffering from a wide range of afflictions such as fungal diseases, various inflammations, and diseases of the liver, heart and blood vessels.

More attention was brought with the recognition that Resveratrol was present in white wine at vanishingly low concentrations, but this was true for all of the flavonoids and polyphenols shown to have desirable biological properties. It was therefore assumed that red wine had to be much more beneficial than white wine. Proponents of red wine consumption made themselves heard, and subsequently the

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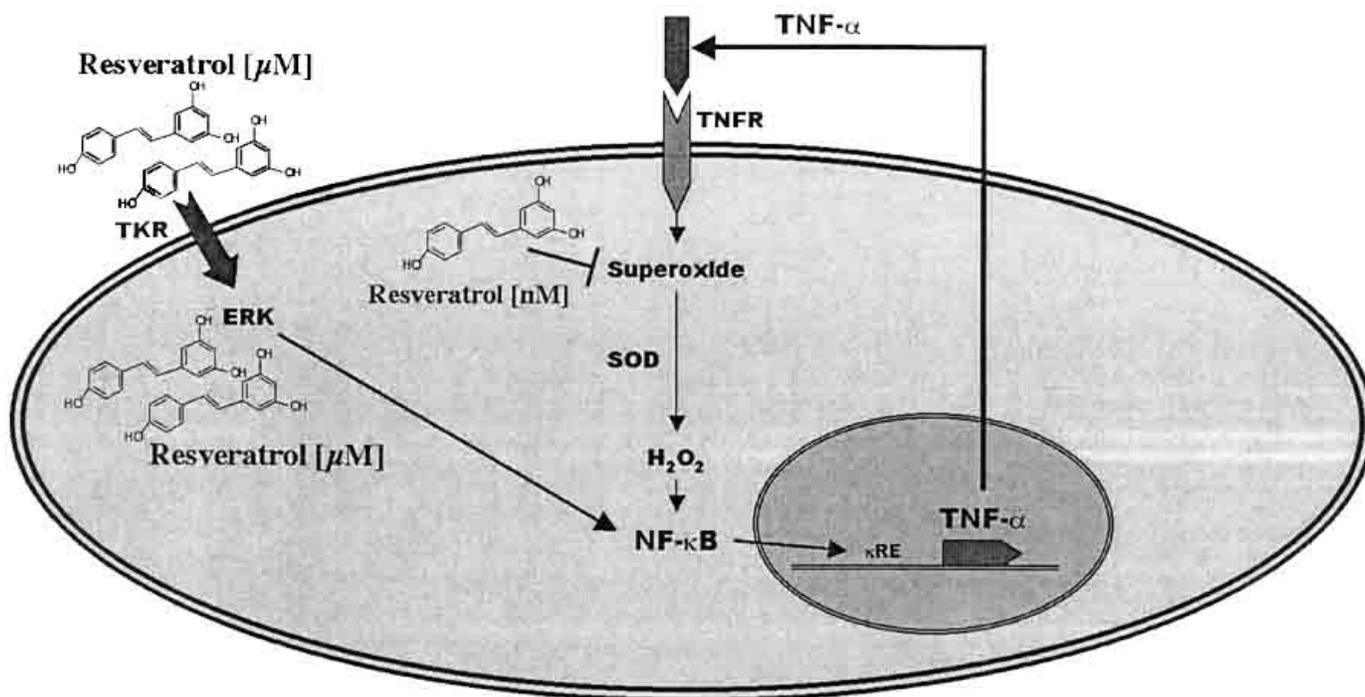


Fig. (1). Schematic representation of the biphasic molecular control of gene expression by Resveratrol. In the nano molar (nM) range concentrations, Resveratrol acts as a potent antioxidant inhibiting the H₂O₂-dependant activation of NF-κB by scavenging the TNF-α-generated superoxide radicals interfering with the TNF-α-related autocrine/paracrine loop. However, in the micro molar (μM) range concentrations, Resveratrol interacts as an agonist or antagonist of cellular receptors and proteins that can activate signal cascades leading to the direct activation of NF-κB (i.e. ERK1/2) and consequently activating the TNF-α-related autocrine/paracrine loop of gene expression.

claims were supported by a number of experimental reports on animal and human investigations providing credence to their view [92, 144, 170, 141].

Goldberg [57, 63] described the concentration of Resveratrol isomers in wines from various well-recognized wine-producing areas of the world. Resveratrol is found primarily in the skin of grapes and is relatively abundant in red, but not white, wines. There is considerable variation in the Resveratrol content even among red wines, depending on grape cultivar, vintage, and place of origin and also on the analytical technique used for measurement. These investigators have analyzed to the present time more than 2000 individual products [57, 61, 63]. Low concentrations of Resveratrol were detected in virtually all white wines and fortified dessert wines. The values rarely exceeded 0.1 mg/L, an exception being for certain German Riesling wines in which the concentrations of *trans*-Resveratrol ranged from 0.75-1.22 mg/L. Among red wines, those of Burgundy and Oregon (where Pinot Noir is the predominant or exclusive cultivar) had the highest concentrations, with those of Bordeaux, the Rhone Valley and Canada next in line. Wines produced in regions notable for warm and dry climates such as California, Australia, Spain, Italy, South Africa and South America, had the lowest Resveratrol concentrations. In general, there was a significant positive correlation between the *trans*- and *cis*-Resveratrol concentrations of all wines. In addition to free Resveratrol, wines contain Resveratrol glycosides that may contribute to the biological response [58].

Resveratrol has been found in at least 72 plant species (distributed in 32 genera and 12 families), and in a number of the human diet, such as mulberries, peanuts, and grapes. Relatively high quantities are found in the latter, possibly because of the response of *Vitis vinifera* (Vitaceae) to fungal infection [31]. Fresh grape skin contains about 50 to 100 mg of Resveratrol per gram, and its concentration in red wine is in the range of 1.5 to 3 mg/liter [85]. Appreciable amounts are also found in white and rosé wines [138].

Oral administration of 28 μg Resveratrol of red wine to male rats resulted in peak plasma levels of Resveratrol of greater than 20 ng/ml after 1h [175]. Importantly, Resveratrol was shown to be bioavailable to several tissues, including heart, liver, and kidney, and was retained in these tissues [175]. Unfortunately, no data have been published concerning serum levels of Resveratrol after wine consumption, and there is no information about its rate of clearance from the bloodstream, the identities and activities of its metabolic products, or the potential first-pass effects of portal circulation through the liver. In the absence of such data, the physiological significance of Resveratrol in wine remains uncertain.

Resveratrol is sparingly soluble in water. Turner administered Resveratrol in a 10% ethanol solution to model mice [175]. The daily intake of ethanol in this study ranged from 0.75-1.5 g/kg, which is equivalent to a 50 kg human consuming 3-6 glasses of wine. This quantity of ethanol is generally considered to exceed moderation [86]. However,

this volume of ethanol is often consumed by non-alcoholics, was essential to solubilize the highest dose rates of Resveratrol, and had no significant effect on any measurement performed in this study.

Resveratrol synthesis from p-coumaroyl CoA and malonyl CoA is induced by stress, injury, infection or UV-irradiation. Resveratrol is classified as a phytoalexin anti-fungicide conferring disease resistance in the plant kingdom.

In the laboratory, its synthesis can be accomplished by the Wittig reaction linking two appropriately substituted phenols through a styrene double bond as described initially by Moreno-Manas and Pleixats [114], and subsequently modified by others [85, 59]. In this strategy, methylated precursors are used to protect the OH groups, which are subsequently removed by boron tribromide with the formation of the *trans*-isomer, which is virtually the only naturally occurring isomer of Resveratrol. These difficult chemistry reactions became unnecessary, as Resveratrol is now commercially available (Sigma, Cat No. R5010) (See Fig. 1).

II. GENERAL PROPERTIES

A. Relationship to Estrogen

Based on its structural similarity to diethylstilbestrol, a synthetic estrogen, Gehm [54] examined whether Resveratrol might be a phytoestrogen. At concentrations (~3-10 μM) comparable to those required for its other biological effects, Resveratrol inhibited the binding of labeled estradiol to the estrogen receptor and it activated the transcription of estrogen-responsive reporter genes transfected into human breast cancer cells. This transcriptional activation was estrogen receptor-dependent, required an estrogen response element in the receptor gene, and was inhibited by specific estrogen antagonists. In some cell types (e.g., MCF-7 cells), Resveratrol functioned as a superagonist (i.e., produced a greater maximal transcriptional response than estradiol) whereas in others it produced activation equal to or less than that of estradiol. Resveratrol also increased the expression of native estrogen-regulated genes, and it stimulated the proliferation of estrogen-dependent T47D breast cancer cells. The estrogenic actions of Resveratrol broaden the spectrum of its biological actions and may be relevant to the reported cardiovascular benefits of drinking wine. The estrogenic effects of Resveratrol (3-10 μM) occur at concentrations that are similar to those required for its reported anti-inflammatory [90, 91], anti-platelet [126], and anti-carcinogenic [84] activities. Data suggest that Resveratrol exerts an antiproliferative effect in Ishikawa cells, and the effect may be mediated by both estrogen-dependent and independent mechanisms.

The growth of estrogen-dependent T47D breast carcinoma cells was also suppressed by Resveratrol treatment [12]. The latter finding is contradictory to the results of previous work by Gehm [54], who demonstrated stimulation of T47D cell proliferation by Resveratrol through interaction with estrogen receptor. The exact reason for this discrepancy remains unknown. In another study,

Resveratrol caused estrogen-like induction of prolactin secretion in the immortalized pituitary cell line PRI, but it exhibited neither estrogen receptor binding nor growth stimulatory activity in these cells [158].

Although Jang [84] found that Resveratrol exerts an anticarcinogenic effect in mouse mammary cultures, the results of Gehm [54] suggest that Resveratrol could exert a growth-stimulating estrogenic effect on human breast carcinoma T47D. This apparent contradiction might be explained by the observation that, although many human breast cancers are mitogenically stimulated by estrogen, most mouse mammary cancers are estrogen-insensitive [122]. Although the anti-carcinogenic and anti-thrombotic activities of Resveratrol show pharmacological promise, its estrogenic properties may produce undesirable side effects and limit the circumstances under which it can be used safely.

Although considerably less potent than estradiol, Resveratrol produced a maximal level of induction that was 2- to 3- fold greater than that achieved by using maximal doses of estradiol (0.1 nM). This superagonism by Resveratrol was highly reproducible and was not observed with DES, which produced the same level of activation as estradiol at saturating (1 nM) concentrations [54]. Suboptimal doses of estradiol and Resveratrol were additive, but maximal activation by Resveratrol was not increased further by estradiol [54]. These results are consistent with both compounds activating the same receptor.

Resveratrol (10-30 μM) was as effective as the maximal (1 nM) dose of estradiol in activating progesterone receptor gene expression [54]. Resveratrol and estradiol also induced expression of the pS2 gene in MCF-7 cells, and both of these estrogen-responsive genes were stimulated in T47D cells.

Turner's *in vivo* results demonstrate that Resveratrol does not stimulate indexes of uterine growth and differentiation in immature rats; even very high doses of Resveratrol generally had insignificant effects on uterine wet weight, epithelial cell height, and insulin-like growth factor-1 (IGF-I) gene expression [175]. Additionally, Resveratrol had no effect on other estrogen target tissues; treatment with Resveratrol did not alter cortical bone growth, the serum concentration of cholesterol, or body weight. They concluded from these findings that Resveratrol is not an estrogen agonist in the rat [175]. The effects of 17 β -estradiol observed in this study were consistent with previous studies. As expected, the hormone stimulated indexes of uterine growth (wet weight) and differentiation (increased epithelial cell height and steady state IGF-I mRNA levels) and suppressed weight gain, serum cholesterol, and radial bone growth [176, 13, 118].

Resveratrol had variable effects on activation of estrogen regulated genes *in vitro*; some were activated to a greater extent than with 17 β -estradiol, whereas others were activated to lesser extent [54]. These findings suggested that Resveratrol might have tissue-selective actions analogous to triphenylethylene- and benzothiophene-selective estrogen receptor modulators [41]. This possibility was not substantiated in the present studies. In contrast to the

selective estrogen receptor modulators, Resveratrol did not reduce serum concentrations of cholesterol, suppress weight gain, or antagonize radial bone growth.

In summary, dose-dependent studies revealed that orally administered Resveratrol had minimal effects on estrogen target tissues in rats, including no effect on uterine growth and differentiation, body weight, serum cholesterol, or radial bone growth. In contrast, Resveratrol antagonized the effects of estrogen to lower serum cholesterol [175]. These findings suggest that it is unlikely that the cardioprotective effects of moderate wine drinking are due to the binding and activation of estrogen receptors by Resveratrol [175].

B. Effects on Lipid Metabolism

One of the earliest studies compared the effects of Resveratrol and polydatin upon the lipid metabolism of rats and mice when given orally and intraperitoneally to animals fed a high cholesterol diet [5]. Both compounds inhibited the deposition of cholesterol and triglyceride in the livers of these animals, as well as decreased the rate of hepatic triglyceride synthesis from [¹⁴C]-acetate.

The effect of Resveratrol on healthy volunteers was tested. These subjects consumed a variety of beverages, each for a 4-week period, namely: white wine with detectable concentrations of Resveratrol, red wine with Resveratrol at a concentration of 4 mg/L, regular commercial grape juice virtually free of Resveratrol, and the latter fortified with Resveratrol to a concentration of 4 mg/L [127, 58]. The consumption of 375 ml of either red or white wine per day led to significant and equivalent increases in the plasma concentrations of high density lipoprotein (HDL)-cholesterol and apolipoprotein A-1 (apoA1), but red wine also caused an increase in the triglyceride concentration, which is a positive risk-factor for coronary heart disease (CHD). Both wines also resulted in equivalent decreases in thrombin-induced platelet aggregation and in plasma thromboxane B₂ concentrations. Grape juice formulations did not alter the plasma lipid or lipoprotein concentrations, and results were paradoxical: the commercial grape juice, which contained very low concentrations of Resveratrol, caused a sharp increase in thrombin-induced platelet aggregation which was dramatically reversed when the volunteers consumed the Resveratrol-enriched juice. These results are not easy to interpret. Comparison of the two wines suggests that as far as lipid metabolism and platelet aggregation are concerned, red wine does not appear to offer any advantage over white wine. This is surprising since the wines differed in their concentrations not only in Resveratrol but also in phenolics that were measured and differed by 10-20 folds. It is highly improbable that the low polyphenols in white wine would have an effect on the observed findings and the observed changes are probably attributable to the alcohol content of the wine. The fact that these changes were not enhanced by red wine is consistent with the notion that alcohol is the dominant biologically active component of this beverage and that the red wine phenolics are either poorly absorbed or fail to augment the already potent effects of alcohol. On the other hand, the difference in thrombin-induced platelet aggregation between commercial and Resveratrol-enriched grape juices

argues in favor of the absorption of this compound in biologically active concentrations by the human subjects, but does not exclude the possibility that this and other effects of Resveratrol which overlap with those of alcohol are not apparent when the latter exceeds a threshold level of consumption. If this idea is correct, it will be difficult to sustain the concept of beverage-specific health effects among moderate alcohol consumers, although the antioxidant potential of red wine and its stimulation of NO synthesis by the vascular endothelium are characteristics which may offer enhanced protection against CHD.

A series of reports [21, 148, 147] described the ability of Resveratrol and of polydatin to inhibit the aggregation of rabbit platelets as well as the formation of thromboxane B₂ from arachidonate. Further, Resveratrol was shown to inhibit the antigen-induced contraction of isolated trachea from guinea pigs rendered sensitive to albumin with an IC₅₀ of 100 mM [134]. This effect was not due to interactions with the histaminergic or cholinergic systems: rather, interaction with beta-blockers, indomethacin and mepacrine suggested that inhibition of arachidonate metabolism was the more likely mechanism.

The effects of Resveratrol on platelet aggregation and cholesterol metabolism are controversial [9, 61, 126, 127, 58, 187]. In current studies in weanling rats, neither ethanol nor Resveratrol altered the total serum cholesterol. However, as 17 β -estradiol reduced total serum cholesterol [13, 152, 181] and Resveratrol antagonized that activity, it was suggested that Resveratrol is an estrogen antagonist on cholesterol metabolism [175].

C. Cardiovascular Effects

Epidemiological studies have shown that the regular consumption of red wine may in part account for the apparent compatibility of a high fat diet with low incidence of coronary atherosclerosis [74]. This phenomenon, commonly referred to as the French paradox, may be associated with wine constituents that exhibit tumor-preventive properties as well as inhibit reactions that increase the risk of coronary heart disease.

Resveratrol, a polyphenol in red wine, induces nitric oxide (NO) synthase, the enzyme responsible for the biosynthesis of NO, in cultured pulmonary artery endothelial cells, suggesting that Resveratrol could afford cardioprotection by affecting the expression of nitric oxide synthase [74].

However, the anti-oxidant [46] and anti-platelet [126, 8] activities of Resveratrol have been invoked as possible mechanisms for the reported cardiovascular benefits moderate wine consumption and the so-called "French paradox" [61]. Goldberg and coworkers [126] found that Resveratrol inhibited thromboxane B₂ synthesis and thrombin-induced aggregation of human platelets *in vitro*, with IC₅₀s of 7 μ M and ~160 μ M, respectively. More recently, they examined the effects of dietary supplementation with Resveratrol and found that platelets from human volunteers who consumed 2 mg (~9 μ mol) per

day showed diminished thromboxane B₂ synthesis and reduced thrombin-induced aggregation compared with controls [127]. A few glasses of many red wines could supply this amount of Resveratrol. These results suggest that daily consumption of some red wines might produce pharmacologically significant concentrations of Resveratrol in the blood. Based on the concentrations required *in vitro*, it seems likely that doses of Resveratrol that affect platelet behavior could also have some estrogenic effect. It is intriguing to consider whether the estrogenicity of Resveratrol may contribute to the reported cardiovascular benefits of red wine [61]. Estrogens have similar effects if administered orally; the high doses delivered to the liver via portal circulation produce beneficial changes in serum lipids [98, 124]. Similarly, wine consumption may expose the liver to higher concentrations of Resveratrol than occur in the systemic circulation.

Some investigators advanced the view that wine drinkers had lower CHD mortality rates than those whose preference lay with other fluids. Wines, especially those of the scarlet hue, are rich in flavonoids and other polyphenolic constituents that have been shown *in vitro* to inhibit platelet aggregation and the synthesis of pro-atherogenic eicosanoids [52, 2, 115]. The consumption of moderate amounts of red wine by human subjects is accompanied by increased blood anti-oxidant activity [106, 184, 48], an event which is likely to diminish in the formation of oxidized low density lipoprotein (LDL), implicated as a potent initiator of atherogenic alterations in the endothelium, subintima and smooth muscle layers of susceptible arteries [159, 40, 188]. But until Resveratrol arrived on the scene, all of the constituents in the phenolic fraction of red wine were merely members of an egregious family widely distributed in plants, fruits and vegetables forming staple components of the human diet [80, 39]. Resveratrol was the unique exception, and wine was the only elixir that gave humanity access to this antidote.

A recent study reported that red wine inhibits endothelin-1 synthesis in cultured bovine aortic endothelial cells by suppressing transcription of the ET-1 gene. The degree of inhibition of ET-1 synthesis correlated with the total polyphenol content [26]. ET-1 is a highly potent vasoconstrictor peptide, and its over production contributes into the development of vascular diseases and atherosclerosis [25]. The finding that small amounts of red wine extract can suppress ET-1 synthesis supports the assertion that moderate intake of red wine can prevent coronary heart disease.

D. Gene Expression: Biphasic Regulation of Gene Expression by Resveratrol

The most relevant biological effect of Resveratrol has been its capability of regulating gene expression. The molecular basis for this regulation is not well understood, not least because multiplicity of studies addressing this specific issue have defined controversial results involving a myriad of possible pathways. The chemical nature of Resveratrol suggests two major ways of biological interaction that might affect the regulation of gene expression in normal and

transformed cells. It is widely accepted that the diphenolic structure of Resveratrol confers a potent antioxidant activity that might affect regulatory pathways sensitive to redox variation. Alternatively, Resveratrol may directly interact with many proteins and receptors posing as an antagonist or agonist in terms of the ulterior functions of the protein with which it interacts.

We have shown that Resveratrol can exhibit a biphasic type of response in the regulation of tumor necrosis factor- α (TNF- α) gene expression in the human prostate cancer cell line PC3. nM concentrations of Resveratrol inhibited the endogenous and TNF- α -induced expression of TNF- α mRNA as determined by semi-quantitative RT-PCR. However, in the high μ M and mM range of concentration, Resveratrol restored and increased the endogenous and the TNF- α -induced expression of TNF- α mRNA in this cells. Moreover, this biphasic response of TNF- α gene expression correlated with both the inhibition and activation of the basal and TNF- α -mediated nuclear factor-kappaB (NF- κ B) following the same pattern of expression of TNF- α [53]. These results suggested the dual role of Resveratrol at the molecular level regulating gene expression. Considering the antioxidant nature of Resveratrol, at low nM and μ M concentrations it might interfere with the H₂O₂-mediated activation of the NF- κ B by decreasing superoxide (O₂⁻) and subsequently lowering the generation of H₂O₂. Similarly, the chemical antioxidant characteristic of nitric oxide (NO) was found to be responsible for the inhibition of the H₂O₂-mediated activation of NF- κ B [53] (Fig. 1).

It has been shown that Resveratrol inhibits the expression of the inducible nitric oxide synthase (iNOS) by down-regulation of NF- κ B binding activity via blockade of I κ B degradation [174]. In addition, quercetin and Resveratrol at μ M range suppressed iNOS gene expression and NO production from LPS and IFN- γ -treated murine macrophages RAW 264.7 [20]. Endothelium-derived generation of TNF- α -induced vascular cell adhesion molecule-1 (VCAM-1) was inhibited by the use of Resveratrol in concentrations present in human plasma following moderate wine consumption ranging between high pM and low nM concentrations [7]. Recently, Resveratrol has been shown to block the TNF- α -induced activation of NF- κ B in cultured U937, Jurkat and HeLa cells. However, in this study, the mechanism by which Resveratrol interferes with the activation of NF- κ B is by inhibiting the phosphorylation and nuclear translocation of the p65 subunit of NF- κ B. NF- κ B activation induced by other stimuli, including tissue-type plasminogen activator (TPA), lipopolysaccharide (LPS), Okadaic acid, ceramide and H₂O₂, was also blocked by Resveratrol [104].

In contrast, Resveratrol at μ M concentrations induced phosphorylation of extracellular signal-regulated kinases, ERK1 and ERK2 in SH-SY5Y human neuroblastoma cells [87]. Recently, Resveratrol has been shown to act as an estrogen agonist and stimulates ERE-driven reporter gene activity in CHO-K1 cells expressing either ER α or ER β . Further, Resveratrol binds ER α and ER β with comparable affinity, but 7000-fold lower affinity than the natural binding molecule estradiol (E₂) [14]. These findings indicated the possible role of Resveratrol inducing the activation of direct

kinase pathways leading to the immediate activation of NF- κ B and others transcription factors that affect gene expression in tumor cells (Fig. 1).

III. ANTICANCER EFFECTS

A. Chemoprevention

A new strategy in the fight against cancer is chemoprevention, which is the prevention or reduction of cancer risk by ingestion of natural or synthetic chemicals with low toxicity that are able to suppress, delay, or reverse carcinogenesis [4].

The phytoalexin Resveratrol has been shown to inhibit cancer initiation, promotion, and progression [84]. This is partly attributable to its antioxidant activities and its inhibition of cyclooxygenase, COX1 and COX 2 [84, 163]. Resveratrol is found in a multitude of dietary plants including grapes and peanuts [84]. The high levels of Resveratrol in grape skin are synthesized in response to fungal infections. Resveratrol is also able to forestall these infections. Grape juice is a good source of Resveratrol as well as wine, which has some particularly high concentrations (1.5 to 3.0 mg/L) [84].

Epidemiological studies have suggested that nutrition plays an important role in carcinogenesis and that 30% of cancer morbidity and mortality can potentially be prevented with proper adjustment of diets.

A newer dimension in the management of neoplasia is increasing awareness that chemoprevention, which refers to the administration of chemical agents to prevent the initiation and promotion events associated with carcinogenesis, and could be the most direct way to reduce mortality and morbidity. In the search for new cancer chemopreventive agents over the past few years, hundreds of plant extracts have been evaluated. Resveratrol, a phytoalexin found in grapes, fruits, and root extracts of the weed *Polygonum cuspidatum*, has been an important constituent of Japanese and Chinese folk medicine [123, 94, 155].

The chemopreventive activity of Resveratrol could be explained by the induction of CD95-dependent apoptotic cell death in tumor cells, resulting in initiation and progression [22]. Several recent communications have highlighted the role of the CD95-CD95L system in drug-induced or immune-mediated clearance of tumor cells [47, 117, 51, 108, 173, 136], suggesting that the fate of antitumor therapy might be determined by the balance between CD95 and CD95L expression on tumor cells and on immune cells. Once triggered the CD95 receptor can then activate a series of intracellular events culminating in the death cascade composed of intracellular caspases [119, 95]. These observations have led to the suggestion that autocrine and/or paracrine CD95/CD95L-mediated signaling might be one critical event in drug-induced cell death. Clement (1998) suggested that Resveratrol treatment provides the critical level of expression of the CD95-CD95L system on tumor cells sufficient to trigger intracellular apoptotic cascade. In addition, the inability of Resveratrol to induce CD95-

mediated cell death in normal PBLs further supports the specific involvement of the CD95-CD95L system in Resveratrol-induced tumor cell death. Resveratrol-induced enhancement of CD95L on CD95⁺ tumor cells constitutes an effective system for the induction of tumor suicide without nonspecific toxicity to the normal peripheral blood lymphocytes (PBLs). The specific involvement of CD95-CD95L system in HL60 cell death induced by Resveratrol is further supported by significant inhibition of cell death in the presence of either anti-CD95 or anti-CD95L, whereas isotype control antibody has little effect on cell death [22].

Similar to HL 60 cells, T47D cells constitutively express the CD95 receptor but not CD95L [22]. However, following 18h of exposure to Resveratrol there is a significant increase in cell surface expression of CD95L, whereas CD95 expression essentially remains unchanged. Concomitantly, 37% of Resveratrol-treated (32mM) T47D cells undergo cell death, which is completely inhibited in the presence of anti-CD95 antibody.

Resveratrol has minimal toxicity to normal PBLs. Moreover, normal human PBLs that express basal levels of CD95 and CD95L do not significantly undergo change in surface expression of either CD95 or CD95L following Resveratrol exposure for up to 72h [22]. The inability of Resveratrol to induce cell death in normal human PBLs, unlike HL60 and T47D cells, could then be explained by the failure of Resveratrol to upregulate CD95L expression on normal PBLs. These results show that Resveratrol-induced cell death is tumor specific and further support the involvement of CD95-CD95L system as the apoptotic trigger.

However, to extrapolate *in vitro* findings to chemoprevention, more studies need to be performed to determine the stability, half-life, and biological significance of the concentrations of Resveratrol needed to activate the apoptotic pathway. Nevertheless, these data provide evidence that this natural chemopreventive agent, not known for its chemotherapeutic potential, also fulfills two basic criteria for an effective therapeutic agent, i.e., tumor specificity and minimal toxicity to the normal hematopoietic cells. Taken together, our findings strongly suggest that Resveratrol merits further investigation as a cancer chemopreventive as well as chemotherapeutic agent in humans.

B. Inhibition of Cell Proliferation

Resveratrol inhibits the proliferation of pulmonary artery endothelial cells, which, based on flow cytometric analysis, correlates with the suppression of cell progression through S and G2 phases of the cell cycle [73] (Fig. 2). In the presence of Resveratrol, the cells accumulate in S and G2-M phases of the cycle. An increase in the proportion of S phase cells, from 8 to 14% and of G2 cells from 4 to 9% was observed at 10 μ M concentration of Resveratrol [73]. A much more dramatic effect was evident at 50 μ concentration, where the proportion of S phase was increased to 35% and of G2 to 42%, concomitant with a drop in proportion of G1 cells from 82% to control level of 16% [73]. Resveratrol, at concentrations comparable with those found in wines and

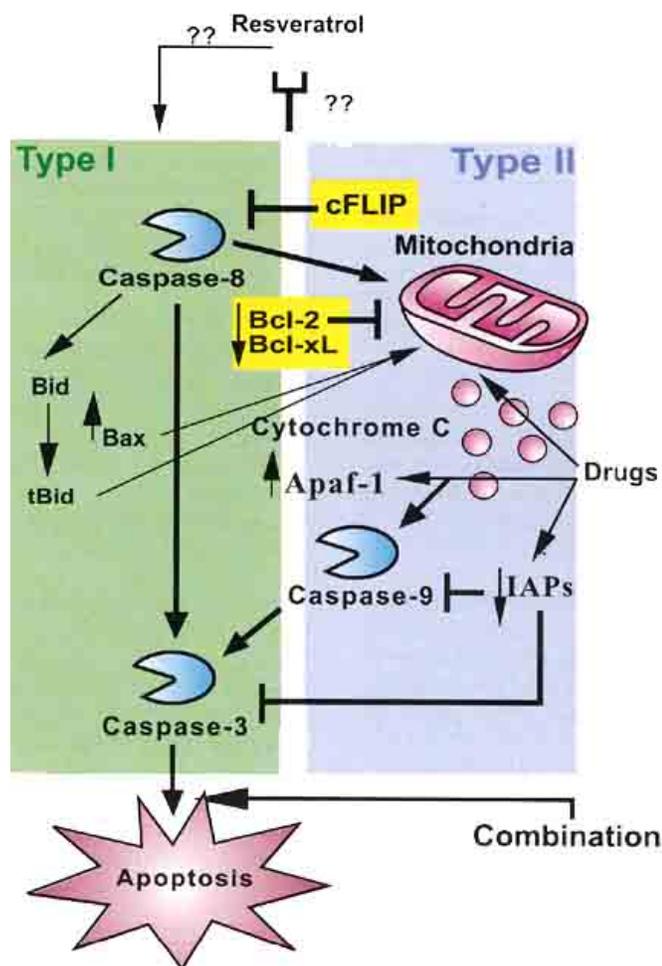


Fig. (2). Schematic diagram of Resveratrol-mediated inhibition of proliferation of cancer cells. After entering the cell via either a receptor-mediated pathway or direct infusion through the plasma membrane, by affecting the normal dynamics of DNA, Resveratrol triggers the p53 protective pathway. As a result, the GADD45 (implicated in DNA repair) and p21^{WAF1} will be induced, both of which will affect cdc2 (cyclin-dependent kinase-2). Cdc2 is essential for cell cycle progression through the G2/M phase and its diminished activity results in cell cycle arrest. Alternatively, Resveratrol, via the p53/p21 pathway can affect specific cyclins and cyclin-dependent kinases of the G1 and S phases (CDK2, 4, 6 and cyclins D, E) of the cell cycle. This will result in increased phosphorylation of the Rb protein, which causes its association with and inhibition of E2F transcriptional activity. The cells will subsequently become arrested at the G1→S transition phase.

grapes [155, 84], effectively suppress endothelial cell proliferation and induces eNOS [74].

Western blot analysis and immunocytochemical protein detection combined with multiparameter flow cytometry further demonstrate that the perturbed progression through the S and G2 phases is accompanied by an increase in the expression of the tumor suppressor gene protein p53 and elevation of the level of cyclin-dependent kinase inhibitor p21^{WAF1/CIP1} [74]. The up-regulation of p53 is, most likely, responsible for the transcriptional induction of p21^{WAF1/CIP1}. The latter is the key inhibitor of the cell cycle progression machinery arresting the cells at check points, including the G2 checkpoint [15] to allow for repair of the DNA damage. Its up-regulation in BPAE cells by Resveratrol, as seen presently, is in all probability directly responsible for inhibiting the cyclin-dependent kinase complexes operated

by Cdk2 and Cdc2 (Cdk1), and there by for the suppression of cell transit through S and G2.

Resveratrol inhibited bovine pulmonary artery endothelial cell proliferation in a time- and concentration-dependent manner, with 10 μ M reducing growth by 30%, and 50-100 μ M completely preventing cell proliferation after a 3-day treatment with the polyphenol [73]. When examined by phase contrast microscopy, control cells displayed the characteristic cobblestone-like growth patterns typical of these cells maintained in culture [125, 129]. In contrast, cells treated with 50-100 μ M Resveratrol assumed a long, spindle-shaped morphology in the tissue culture flasks, which, following trypsinization and resuspension in phosphate buffer saline (PBS), appeared under the microscope to be dominantly as giant cells, compared with controls [73].

It is evident from the DNA content frequency histogram as well as from p53 versus DNA, or p21 versus DNA scatter plots representing these cultures, that most cells were arrested in the late portion of S phase [73]. That is, the proportion of cells with DNA index 1.4-1.9 was about 4-fold greater than of the cells with DNA index between 1.1 and 1.3 [73].

The presence of cells with fractional DNA content, which is a characteristic feature of apoptosis [28], was also apparent in the Resveratrol-treated cultures. Their frequency was increasing with the increase in concentration of Resveratrol, from about 2% in control to 4, 7 and 20% at 10, 50, and 100 μM Resveratrol, respectively [75].

The cell arrest in S and G2-M, the increase in p53 and p21^{WAF1/CIP1}, all were observed at 50-100 μM concentrations [74]. However, although the absolute increase in p53 was most pronounced for cells in S and G2-M phase, the increase was of similar magnitude when recalculated per unit of DNA, regardless of the cell cycle position.

Since Resveratrol has been reported to significantly inhibit ribonucleotide reductase [44], an enzyme that affects DNA replication indirectly by regulating the availability of DNA synthesis precursors, it could provide a mechanistic basis for the observed prolongation of S phase in Resveratrol-treated cells.

Since nm23 has been reported to display anti-metastatic activities [93, 49, 142], it was reasoned that its overexpression may provide some insight on the relationship between Resveratrol and control of metastasis [73]. The results showed that the catalytic activity of nm23 per se is not involved in the observed cellular response [73]. Furthermore, treatment with Resveratrol had no discernible effect on nm23 expression.

Resveratrol is a remarkable inhibitor of ribonucleotide reductase [44]. It is much more effective than hydroxyurea, hydroxyanisole, the only ribonucleotide reductase tyrosyl radical scavengers used in clinics, and the potent p-propoxyphenol [131]. The most active radical scavenger described up to now acting on ribonucleotide reductase is 5-amino-1-formyl isoquinoline thiosemicarbazone [171, 96]. However, thiosemicarbazones have no application as drugs, because of their very strong toxicity. Fontecave suggests that Resveratrol should thus be considered as an alternative to the more toxic hydroxyurea, for example in studies of biotherapeutic approaches against HIV [44].

C. Apoptosis

The role of p53, in addition to induction of p21^{WAF1/CIP1}, is also in protection of the genome integrity via physical interaction with DNA, as well as in regulation of cell propensity to apoptosis. The latter function was shown to involve the induction of expression of the apoptosis-promoting gene bax [112]. High level of p53 expression in the cells with fractional DNA content, i.e. in apoptotic cells, strongly suggests that their apoptosis may be associated with up-regulation of p53.

Resveratrol suppresses tumor promoter-induced cell transformation and markedly induces apoptosis, transactivation of p53 activity and expression of p53 protein in some cell lines and at the same concentration [78]. Also Resveratrol-induced apoptosis occurs only in cells expressing wild type p53 ($p53^{+/+}$), but not in p53-deficient ($p53^{-/-}$) cells, while there is no difference in apoptosis induction between normal lymphoblasts and sphingomyelinase-deficient cell lines. These findings suggest that Resveratrol induces apoptosis through activation of p53 activity, suggesting that its anti-tumor activity may occur through the induction of apoptosis.

Apoptosis results from activation of a pre-programmed pathway of biochemical events leading to cell death [43, 190]. A large body of evidence indicates that apoptosis may represent a protective mechanism against neoplastic development by eliminating genetically damaged cells or excess cells that improperly have been induced to divide by factors such as carcinogens [6, 68, 145, 120, 82, 45, 178]. *In vitro* and studies demonstrate that suppression of apoptosis is involved in tumor promotion by chemical agents [154, 16, 137, 172]. Sphingomyelinase (SMase)-deficient lymphoblast line, MS 1418 and a normal lymphoblast control cell line used to determine whether ceramide/SMase is involved in Resveratrol-induced apoptosis, Resveratrol induced apoptosis in both cell lines. These data rule out the involvement of ceramide/SMase in Resveratrol-induced apoptosis.

All of the above-observed effects of Resveratrol, including induction of apoptosis, were used at high concentrations, are also compatible with the putative chemopreventive and/or anti-tumor activity of Resveratrol [75].

Suppression of apoptosis may be a failure of tumor promotion by chemical carcinogenesis. Indeed, many chemopreventive agents may act through the induction of apoptosis as a mechanism of anticarcinogenesis.

TNF induced ten fold activation of NF- κ B, and Resveratrol inhibited this activation in a dose-dependent manner; full inhibition occurred at 5 $\mu\text{g/ml}$ resveratrol [104]. Resveratrol even at 25 μM by itself did not activate NF- κ B. They next examined the effect of changes in the length of incubation with resveratrol on NF- κ B activation by TNF. Cells were incubated with 5 μM Resveratrol for different times and then stimulated with 0.1 nM TNF for 30 min and assayed for NF- κ B. Resveratrol inhibited TNF-induced NF- κ B activation with increased time of incubation.

TNF-induced cytotoxicity and caspase activation was blocked by Resveratrol. Because NF- κ B activation has been shown to play an anti-apoptotic role, the suppression of apoptosis by Resveratrol may seem paradoxical. Resveratrol blocks TNF-induced reactive oxygen intermediate (ROI) generation and lipid peroxidation and this may explain the mechanism by which Resveratrol exerts its effects.

D. Therapeutic Effects

Cancer is the largest single cause of death in both men and women, claiming over 6 million lives each year

worldwide. Chemoprevention, the prevention of cancer by ingestion of chemical agents that reduce the risk of carcinogenesis [156], is one of the most direct ways to reduce morbidity and mortality. The inhibition of COX activity is relevant to cancer chemoprevention because COX catalyzes the conversion of arachidonic acid to pro-inflammatory substances such as prostaglandins, which can stimulate tumor cell growth and suppress immune surveillance [130, 64]. In addition, COX can activate carcinogenesis to forms that damage genetic materials [193, 185].

The process of chemical carcinogenesis can be divided into three general stages, and chemopreventive agents have been categorized according to the stage that they inhibit [180]. Resveratrol inhibits cellular events associated with tumor initiation, promotion, and progression. Resveratrol inhibits the cyclooxygenase activity of COX-1 (median effective dose $ED_{50}=15\ \mu\text{M}$), and this activity correlates with anti-tumor promotion. Resveratrol-mediated inhibition was specific for the cyclooxygenase activity of COX-1 because there was no discernable activity when oxygen uptake was assessed with COX-2, an inducible form of the enzyme associated with responses such as inflammation [55], and inhibition of hydroxyperoxidase activity of COX-2 ($ED_{50}=85\ \mu\text{M}$) was greatly reduced relative to the activity observed with COX-1. Jang (1997) detected that Resveratrol has anti-inflammatory activity both in acute and chronic phase in rats. Overall, these data demonstrate the potential of Resveratrol to inhibit tumor promotion.

Resveratrol was found to inhibit events associated with tumor initiation. For example, Resveratrol inhibited, in a concentration-dependent manner, free-radical formation ($ED_{50}=27\ \mu\text{M}$) when human promyelocytic leukemia (HL60) cells were treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) [149]. Resveratrol inhibited the mutagenic effect of 7,12-dimethylbenz(a)anthracene (DMBA) on the *Salmonella typhimurium* strain TM677 ($ED_{50}=4\ \mu\text{M}$) [146].

Resveratrol inhibited the progression stage of carcinogenesis in the HL 60 cells [164]. Under normal culture conditions, these cells have unlimited proliferative capacity. In a concentration dependent manner, Resveratrol induced expression of nitroblue tetrazolium reduction activity, a marker of granulocyte formation ($ED_{50}=11\ \mu\text{M}$), and nonspecific acid esterase activity, a marker of macrophage (monocyte) formation ($ED_{50}=19\ \mu\text{M}$). Concurrently, incorporation of [^3H] thymidine was inhibited ($ED_{50}=18\ \mu\text{M}$), indicative of terminal differentiation to a nonproliferative phenotype.

Resveratrol inhibited, in a dose-dependent manner, the development of DMBA-induced preneoplastic lesions ($ED_{50}=3,1\ \mu\text{M}$) in a mouse mammary gland culture model of carcinogenesis [84]. No signs of toxicity were observed, as judged by morphological examination of the gland [84]. This effect of Resveratrol shows its cancer chemopreventive activity. The authors studied tumorigenesis in the two-stage mouse skin cancer model in which DMBA was used as initiator and TPA as promoter [84]. During an 18-week study mice treated with DMBA-plus TPA developed an average of two tumors per mouse with 40% tumor incidence. Application of 1, 5, 10 or 25 μmol of Resveratrol together

with TPA twice a week for 18 weeks reduced the number of skin tumors per mouse by 68, 81, 76 or 96%, respectively, and the percentage of mice with tumors was lowered by 50, 63, 63, or 88% respectively. No overt signs of Resveratrol-induced toxicity were observed, as judged by visual inspection of the skin, gross morphological examination of major organ systems, or change in body weights, relative to controls.

The effect of Resveratrol on the human colonic adenocarcinoma cell line Caco-2 was studied. The compound inhibited cell growth and proliferation of Caco-2 cells in a concentration-dependent manner (12.5-200 $\mu\text{mol/L}$). These findings suggest that Resveratrol exerts chemopreventive effects on colonic cancer cells by inhibition of the cell cycle. They 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 the highly metastatic Lewis lung carcinoma (LLC) tumors, but not at a dose of 0.6 mg/kg .

Resveratrol could be a promising anticancer agent for both hormone-dependent and hormone-independent breast cancers, and may mitigate the growth stimulatory effect of linoleic acid in the Western-style diet. The data show a direct inhibitory effect of low concentrations of antioxidant wine phenols on the proliferation of human prostate cancer cell lines mediated by the production of NO, further suggesting the potential beneficial effects of wine and other phenol-containing foods or drinks for the control of prostate cancer cell growth.

Resveratrol inhibits tumor promoter (TPA or EGF)-induced JB6 C1 41 cell transformation in a dose dependent manner in the range 2.5-40 μM . This concentration range is relevant regarding possible biological effects of Resveratrol in daily consumption of grape beverages [31, 85, 62, 138, 126].

Huang (1999) used a mouse JB6 epidermal cell line, a well-developed cell culture model for studying tumor promotion [76, 79, 35, 24, 76], to investigate the molecular mechanism of the chemopreventive effect of Resveratrol.

Inhibition of apoptosis is one mechanism of tumor formation and many chemopreventive agents may act through the induction of apoptosis to block the carcinogenic process [16, 137, 172, 143, 17, 89, 43, 190]. Treatment of cells with Resveratrol induced apoptosis in C1 41 cells in the same dose range that inhibited cell transformation. These results support our notion that the cancer preventive effect of Resveratrol may occur through the induction of apoptosis. However, the cytotoxic effect observed is not paralleled by results obtained *in vitro*. In fact, after 24h culture in the presence of Resveratrol the AH-130 cell number is comparable to controls, and no occurrence of apoptosis is detectable either morphologically or by flow cytometry [18]. The lack of toxicity on cultured AH-130 hepatoma could be related to the low proliferative rate shown by these cells *in vitro*. Another possibility is that the *in vivo* observations are due to an indirect pathway, which may involve molecules produced by the host. Apoptotic cell death is frequently associated with decreased activation of NF- κ B transcription

factor [191]. It has been shown that eicosanoids, and in particular prostaglandin-E₂ (PGE₂), are strong activators of this factor [189, 27]. Since Resveratrol is a cyclooxygenase inhibitor [126, 84, 163] it could be speculated that, reducing the host eicosanoid production, it may cause a decrease in NF- κ B activation, enhancing the apoptotic cell death; indeed, such an inhibitory effect has been demonstrated [37]. On the other hand, it may be that Resveratrol administration to tumor bearing rats causes an increase of CD95L levels [22], which may be involved in AH-130 cell death. These data suggest that Resveratrol can be effective not only in the chemoprevention of tumor development, but also as chemotherapeutic agent.

Resveratrol inhibits protein kinase C (PKC), a key mediator of tumor promotion stage of carcinogenesis [161]. Resveratrol has a broad range of inhibitory potencies against purified PKC that depend on the nature of the substrate and the cofactor dependence of the phosphotransferase reaction [161]. Resveratrol inhibits PKC isozymes with divergent regulatory domains with comparable efficacy. Resveratrol is a catalytic domain-directed PKC inhibitor [161]. Potent inhibition of PKC-catalyzed protamine sulfate phosphorylation by Resveratrol through a distinct kinetic mechanism [161].

The majority of adult cancers are carcinomas of epithelial origin with lung, colon, and uterus as the primary sites, which reflects a selective vulnerability of these tissues to carcinogenic insult as a result of frequent exposure to external environment. Indeed, it is estimated that up to 80 to 90% of all cancers are attributable to environmental risk factors, including chemicals, radiations, and viruses. The notion that a majority of human cancers have an environmental origin implies an optimistic outlook in terms of cancer prevention since most cancer-causing substances are introduced into the environment by human activities and are hence, controllable or removable. The elimination of environmental carcinogenesis or at least avoiding exposure to them offers the opportunity to prevent most cancers, which is a basis of primary prevention.

Prostaglandins are known to play pivotal roles in pathogenesis of malignancy, particularly in colon and mammary carcinogenesis and suppression of prostaglandin biosynthesis through selective inhibition of COX is hence, now regarded as an important cancer chemopreventive strategy. Resveratrol was shown to inhibit COX-1 activity in microsomes derived from sheep seminal vesicles [195]. More recently, Subbaramaiah [163] have reported that Resveratrol inhibits the catalytic activity of inducible isozyme COX-2 in cultured human mammary epithelial cells with and without TPA treatment. Besides inhibiting the catalytic activity of COX-2, the compound also blocked TPA-mediated induction of *cox-2* mRNA in cultured human mammary epithelial cells through repression of AP-1 dependent transactivation [163].

The relation between diet and cancer has interested scientists almost ever since the disease was discovered. Indeed, diet is important factor involved in the development of almost 40% of all neoplastic disease in humans [33], either because of the presence of carcinogens in foods or because of the low assumption of foods rich in substances

with postulated cancer preventive properties such as many antioxidant molecules [29, 139].

Resveratrol administration to rats inoculated with a fast growing tumor (the Yoshida Ah-130 ascites hepatoma) caused a very significant decrease (25%) in the tumor cell content [18]. The effects of this diphenol were associated with an increase in the number of cells in the G2/M cell cycle phase [18]. Interestingly, flow cytometric analysis of the tumor cell population revealed the existence of an aneuploid peak (representing 28% of total), which suggests that Resveratrol causes apoptosis in the tumor cell population resulting in a decreased cell number [18].

It has previously been demonstrated that administration of dealcoholized wine extracts to transgenic mice that spontaneously develop cancer results in a decrease of tumor incidence [23]. It is therefore conceivable that the tumor-preventing activity exerted by wine may be related to Resveratrol. Moderate wine consumption, thus, could be an important factor to consider when dealing with cancer prevention.

Since metastasis begins with uncontrolled proliferation of malignant cells and subsequent degradation of extracellular matrix at the primary site by proteases these cells secrete, including cathepsin D [56, 83, 121], the effects of resveratrol on the expression of cathepsin D was tested. Since elevated cathepsin D has been associated with increased propensity to undergo metastasis in breast cancer cells [56, 83, 121], up-regulation of its expression by Resveratrol makes it unlikely that Resveratrol could impede metastasis by regulating the expression of matrix proteases such as cathepsin D [73].

Resveratrol has been shown to induce anti-proliferation and apoptosis of human cancer cell lines. In this study, they determined the effect of high intracellular levels of the anti-apoptosis protein Bcl-2 on caspase-3 activation, PLC-gamma1 degradation and cytochrome c release during Resveratrol-induced apoptosis. These findings indicate that Bcl-2 inhibits Resveratrol-induced apoptosis by a mechanism that interferes with cytochrome c release and activity of caspase-3 that is involved in the execution of apoptosis. Resveratrol, a plant antibiotic, has been found to have anticancer activity and was recently reported to induce apoptosis in the myeloid leukemia line HL60 by the CD95-CD95 ligand pathway. However, many acute lymphoblastic leukemias (ALLs), particularly of B-lineage, are resistant to CD95-mediated apoptosis. These results point to a general mechanism of apoptosis induction by Resveratrol in ALL cells that involves a mitochondria/caspase-9-specific pathway for the activation of the caspase cascade and is independent of CD95-signaling. The mechanism by which Resveratrol imparts cancer chemopreventive effects is not clearly defined. Here, Resveratrol treatment of the cells causes an induction of WAF1/p21 that inhibits cyclin D1/D2-cdk6, cyclin D1/D2-cdk4, and cyclin E-cdk2 complexes, thereby imposing an artificial checkpoint at the G(1)->S transition of the cell cycle. These data strongly suggest that both ERKs and p38 kinase mediate Resveratrol-induced activation of p53 and apoptosis through phosphorylation of p53 at serine 15.

E. Sensitization Effects of Resveratrol

Accumulating evidence suggest that treatment of various cancer cell lines with Resveratrol induces apoptosis in a dose-dependent manner at concentrations greater than 10 $\mu\text{g/ml}$ [78, 169, 150, 102, 128]. Resveratrol has been shown to affect the expression levels and activity of certain pro- and anti-apoptotic gene products. Some studies emphasize that the apoptotic effects of Resveratrol are dependent on p53 activity [78] and the expression of p53-regulated gene products, such as c-myc and Bax [100]. The activation of p53 may involve a variety of kinase signaling pathways, such as extracellular signal-regulated protein kinases (ERK 1/2) and p38 mitogen activated protein kinase (p38 MAPK) [150]. Through the activation of the ERK1/2 signal transduction pathway, Resveratrol is capable of increasing the expression of p53, serine phosphorylation of p53, and induction of p53-dependent apoptosis in papillary and follicular thyroid carcinoma cell lines [151]. An analog of Resveratrol (3,4,5,4'-tetrahydroxystilbene) was shown to induce p53, which led to increased expression of Bax [100], a pro-apoptotic member of the bcl-2 family of proteins which affects mitochondrial stability.

In contrast, other studies have shown that Resveratrol induces apoptosis through a p53-independent mechanism involving the mitochondrial apoptotic pathway [102]. It was recently shown that Resveratrol is capable of abrogating EGF- and tissue-type plasminogen activator (TPA)-induced ERK 1/2 activation by inhibiting PKC activation, which induced apoptosis in androgen-independent prostate cancer cells [162]. Resveratrol has also been shown to inhibit TNF-induced activation of mitogen-activated protein kinase kinase and c-Jun N-terminal kinase [104]. Through the inhibition of MAPK pathways, Resveratrol has the potential of inducing apoptosis and/or sensitizing tumor cells to drug-mediated apoptosis.

Earlier studies have shown that Resveratrol treatment of human leukemia cell line HL60 increased the expression of Fas ligand, leading to apoptosis through the activation of Fas signaling pathway [22]. This pathway involves the activation of caspase cascade and subsequent cleavage of the DNA repair enzyme, poly(ADP-ribose) polymerase (PARP) [22]. More recent studies have shown that Resveratrol utilizes a CD95-independent apoptotic pathway. This pathway involves the depolarization of the mitochondrial membrane [36], release of cytochrome c, formation of the apoptosome complex and subsequent activation of caspase-9 and -3 [36, 116], and PARP cleavage, which leads to DNA fragmentation and apoptosis. Overexpression of the anti-apoptotic gene products such as Bcl-2 that block cytochrome c release, prevent the progression of the apoptosis signal transduction pathway and caspase 3 activation induced by Resveratrol [128].

Resveratrol is also capable of suppressing the activation of the transcription factor NF- κ B. Resveratrol blocks TNF-induced activation of NF- κ B in myeloid, lymphoid, and epithelial cell lines [104]. The inhibition of I κ B kinase activity [70] by Resveratrol may indicate that this chemical has the ability to disrupt the signaling cascade that leads to

the activation of NF- κ B. Suppression of the NF- κ B activity by Resveratrol may explain the decrease in Bcl-2 expression and subsequent death of the cells [167]. It is likely that the expression of other anti-apoptotic gene products that are under the transcriptional regulation of NF- κ B such as cellular inhibitors of apoptosis protein (c-IAP) family members are also decreased upon Resveratrol treatment.

In addition to the direct induction of the apoptosis, Resveratrol is capable of sensitizing drug-refractory tumor cells to apoptosis induced by chemotherapeutic drugs or immunotherapeutic agents. It has been demonstrated that Resveratrol could sensitize neuroblastoma, glioblastoma, and medulloblastoma cells to TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis through the induction of p21^{WAF-1} and cell cycle arrest at G1, which in turn down-regulated the expression of survivin [1, 50]. In combination with 5-FU, Resveratrol has shown synergistic apoptosis [166].

Recent work from our laboratory has shown that treatment of resistant AIDS-related and non-AIDS related human non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) cell lines with Resveratrol decreased the rate of proliferation. No perturbation of cell cycle distribution was noticed. Resveratrol also induced varying degrees of apoptosis in these cell lines in a dose-dependent manner at concentrations of 10-200 μM .

Since Ramos cells exhibited the most sensitivity to Resveratrol-mediated apoptosis, they were selected for further biochemical analysis. Immunoblot analysis of Resveratrol-treated Ramos cells showed decreases in the amount of pro-caspases-9 and -3, which indicates their activation and cleavage. This was accompanied by PARP cleavage. Truncation of Bid (tBid), the pro-apoptotic member of the Bcl-2 family, was observed, which may indicate that Resveratrol utilizes tBid in the depolarization of the mitochondria and subsequent release of cytochrome c. The expression of IAP family members (c-IAP-1 and c-IAP-2), which act as inhibitors of distal/executioner caspases, was down-regulated by Resveratrol, which may facilitate the progression of apoptosis signaling. Similar results were obtained for the 2F7 AIDS-related lymphoma cells. Therefore, it seems that Resveratrol, by modulating the expression levels of various pro- and anti-apoptotic gene products mainly those involved in the maintenance of mitochondrial stability, facilitates the apoptotic signaling pathway to fully proceed.

We were also interested to examine whether Resveratrol is capable of sensitizing tumor cells to various drugs. Our data indicate that Resveratrol is capable of sensitizing drug-refractory NHL Ramos cell line to various apoptotic-inducing agents in a synergistic fashion. The agents include chemotherapeutic drugs such Gemcitabine, Navelbine, cisplatinum (CDDP), the anti-microtubule drug Paclitaxel, and TRAIL. Resveratrol's ability to sensitize tumor cells to TRAIL has been recently confirmed by another group (Fulda, 2002). However to our knowledge, this is the first demonstration that Resveratrol sensitizes drug-refractory NHL cells to chemotherapy (Fig. 3).

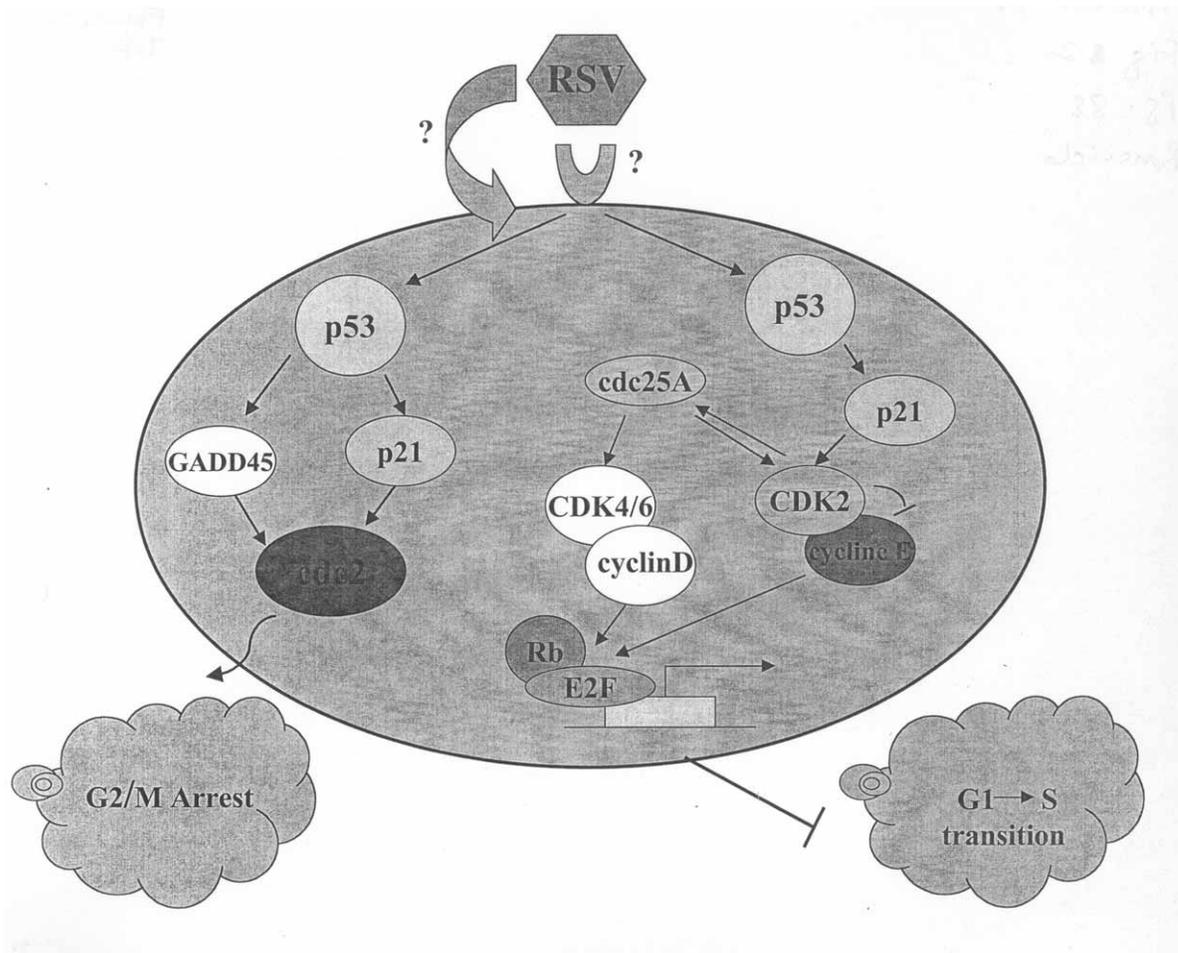


Fig. (3). Schematic diagram of Resveratrol-mediated sensitization of cancer cells to drug-induced apoptosis. Our findings demonstrate that Resveratrol (RSV) is capable of sensitizing drug-resistant tumor cells to the apoptotic effects of chemotherapeutic drugs. The mechanism of RSV-mediated drug-sensitization is schematically shown above. Resveratrol, either via a receptor-mediated pathway or by direct infusion through the plasma membrane, interferes with type I and type II apoptosis signaling pathways [196]. With respect to type I, depending on the amount of caspase-8, Resveratrol either directly induces caspase-8 auto-cleavage/processing (by yet unknown mechanism), which leads to caspase-3 activation and cleavage of death substrates (such as PARP) with subsequent induction of apoptosis. Alternatively, in the event that cells lack sufficient amounts of caspase-8, Resveratrol utilizes type II apoptotic signaling pathway involving mitochondria and the pro-apoptotic bcl-2 family member, Bid, will be cleaved. The caspase-cleaved fragment of Bid (tBid) will then migrate and reside in the mitochondrial outer member, where in collaboration with other pro-apoptotic molecules mainly Bax induce the formation of mitochondrial permeability transition pore (PTP). Resveratrol has also been demonstrated to decrease the expression of anti-apoptosis bcl_{xL}. Decreased levels of bcl_{xL} plus the presence of tBid and increased expression of Bax will alter the cellular ratio of pro- /anti-apoptosis bcl-2 family members and favors apoptosis mediated by drugs. These events will destabilize mitochondria resulting in the release of apoptogenic molecules such as cytochrome c. Resveratrol also upregulates the expression levels of apoptosis protease activating factor-1 (Apaf-1), which in combination with cytochrome c will facilitate the assembly of the apoptosome complex (dATP/ATP-Apaf 1-cytochrome c-caspase 9). Resveratrol can reduce the expression of certain inhibitors of apoptosis protein (IAP) family members, thereby removing the inhibitors of caspase activation. However, these events by themselves are not sufficient for the full induction of apoptosis. The process is facilitated and expedited by the action of drugs, which also modify the expression of apoptosis regulatory gene products. Through auto-catalytic activation caspase-9 becomes processed, and in the absence of natural inhibitors (IAPs) will use caspase-3 (also -6, -7) as a substrate and apoptosis ensues. This is the converging point of type I and II pathways (diagram adopted from unpublished data from our laboratory).

Concluding Remarks

Resveratrol exerts pleiotropic effects on mammalian cells and some of those effects are selected for certain cell types. For instance, in the laboratory and *in vivo* findings suggest that Resveratrol exerts chemotherapeutic and cytotoxic effects against cancer cells. Resveratrol can also sensitize drug-resistant tumor cells to become sensitive to drug-mediated effects. These findings are encouraging for the clinical potential application of Resveratrol in the treatment of human cancer. However, several issues need to be resolved prior to clinical application. For instance, toxicity studies of Resveratrol in experimental animals have not been performed in detail and no toxicity data exists on Resveratrol in humans. Also, many of the reported studies used high concentrations of Resveratrol and it is not known if such high doses can be given to humans to achieve pharmacologically active levels in the circulation. Also, since Resveratrol is highly lipid-soluble, it can be dependent on adipose tissue and other tissues of high lipid content. These and other issues need to be addressed rigorously before any direct therapeutic application. However, the demonstration that Resveratrol used at low subtoxic concentrations can sensitize resistant tumor cells to chemotherapeutic drug-mediated cytotoxicity suggests that non-toxic doses of Resveratrol may be clinically beneficial as a sensitizing agent in combination with other cytotoxics.

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