Cancer Chemopreventive Activity of Resveratrol

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ABSTRACT: Cancer chemopreventive agents are designed to reduce the incidence of tumorigenesis by intervening at one or more stages of carcinogenesis. Recently, resveratrol, a natural product found in the diet of humans, has been shown to function as a cancer chemopreventive agent. Resveratrol was first shown to act as an antioxidant and antimutagenic agent, thus acting as an anti-initiation agent. Further evidence indicated that resveratrol selectively suppresses the transcriptional activation of cytochrome P-450 1A1 and inhibits the formation of carcinogen-induced preneoplastic lesions in a mouse mammary organ culture model. Resveratrol also inhibits the formation of 12-O-tetradecanoylphorbol-13-acetate (TPA)–promoted mouse skin tumors in the two-stage model. The enzymatic activities of COX-1 and -2 are inhibited by resveratrol in cell-free models, and COX-2 mRNA and TPA-induced activation of protein kinase C and AP-1–mediated gene expression are suppressed by resveratrol in mammary epithelial cells. In addition, resveratrol strongly inhibits nitric oxide generation and inducible nitric oxide synthase protein expression. NFκB is strongly linked to inflammatory and immune responses and is associated with oncogenesis in certain models of cancer, and resveratrol suppresses the induction of this transcription factor by a number of agents. The mechanism may involve decreasing the phosphorylation and degradation of IκBα. At the cellular level, resveratrol also induces apoptosis, cell cycle delay or a block in the G1→S transition phase in a number of cell lines. Thus, resveratrol holds great promise for future development as a chemopreventive agent that may be useful for several disorders. Preclinical toxicity studies are underway that should be followed by human clinical trials.

KEYWORDS: resveratrol; cancer chemoprevention; anti-initiation agents; antioxidants; antimutagens

INTRODUCTION

Carcinogenesis may arise as a result of chemical or biological insults to normal cells in a multistep process that involves changes at the genetic level (initiation) followed by promotion and progression that ultimately lead to malignancy. Administration of agents to prevent, inhibit, or delay progression of carcinogenesis has...
been termed chemoprevention. Potential chemopreventive agents that have been or will be evaluated in clinical trials include micronutrients, minerals, or synthetic compounds. Natural products, however, are of particular interest as chemopreventive agents. One important factor is that there may be experience with human consumption. On the basis of bioassay-guided fractionation of plant extracts collected worldwide, recent discoveries of chemopreventive agents in our laboratory include brassinin, deguelin, sulforamate, and resveratrol.

Resveratrol (trans-3,4',5-trihydroxystilbene), originally identified as a phytoalexin by Langcake and Pryce, has attracted considerable attention due to its abundance in grapes and grape products such as wine, a long-standing component of the diet. Epidemiological studies, such as with the French population, have shown an inverse correlation between intake of wine and death resulting from coronary heart disease. Polyphenolics in red wine are suspected to afford these cardioprotective effects due to their spectrum of biological activities, especially as antioxidants. The discovery of resveratrol, a polyphenolic, as a cancer chemopreventive agent has offered renewed interest in grapes and grape products, and dietary supplements based on resveratrol are available. We currently provide an overview of our chemoprevention studies performed with resveratrol, including inhibition of reactive oxygen species (ROS) and cyclooxygenase (COX), and efficacy in skin and mammary animal models of tumorigenesis. Relevant work by others who have investigated the chemopreventive effects of resveratrol is also discussed, as well as estrogen modulatory activities.

**ANTIOXIDANT EFFECTS**

Electron acceptors such as molecular oxygen react easily with free radicals, to become ROS such as $O_2^-$, $H_2O_2$, and $'OH$. These ROS are being continuously generated in cells exposed to an aerobic environment, and have been associated with the genesis of tumors. The damage incurred by proteins and DNA on contact with ROS can modulate carcinogenesis at all three stages, and the chemoprotective role of antioxidants abundant in fruits, vegetables and beverages has received considerable attention. ROS also oxidize low density lipoproteins (LDL) that interact with scavenger receptors for macrophages, inducing the formation of lipid-laden foam cells that contribute to the development of atherosclerotic lesions. Thus, consumption of antioxidants such as vitamin E have been suggested to offer protection against such cardiovascular complications. In a similar manner, as described in a recent review, resveratrol facilitates antioxidant mechanisms. However, bearing in mind the scope of the current article, we will restrict this discussion to the cancer chemopreventive antioxidant mechanisms of resveratrol.

We have shown that resveratrol can inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA)–induced free radical formation with cultured HL-60 cells (IC$_{50}$ 6.2µg/ml). In a DU145 prostate cancer cell line, resveratrol effectively inhibited growth; this was accompanied by a decrease in nitric oxide (NO) production and an inhibition of inducible nitric oxide synthase (iNOS). In a related study, resveratrol was shown to suppress the formation of superoxide radical ($O_2^-$) and $H_2O_2$ produced by macrophages stimulated by lipopolysaccharide (LPS) or phorbol esters (TPA).
Resveratrol also suppressed COX-2 induction, [3H]arachidonic acid ([3H]AA) release and prostaglandin (PG) synthesis, all stimulated by LPA or TPA.²² Manna et al.²² have shown that resveratrol is capable of inhibiting reactive oxygen intermediates (ROI) generation and lipid peroxidation induced by tumor necrosis factor (TNF) in wide variety of cells. In addition, it was reported that resveratrol inhibits unopsonized zymosan-induced oxygen radical production in murine macrophages and human monocytes and neutrophils.²³

In vivo evidence of the antioxidant capacity of resveratrol was also illustrated by protection against renal oxidative DNA damage induced by the kidney carcinogen KBrO₃.²⁴ We have observed that pre-treatment of mouse skin with resveratrol negated several TPA-induced oxidative events in a dose-dependent manner. H₂O₂ and glutathione levels were restored to control levels, as were myeloperoxidase, oxidized glutathione reductase and superoxide dismutase activities. TPA-induced increases in the expression of c-fos and TGF-β1 mRNA were also selectively inhibited.²⁵

**EFFECTS ON CYTOCHROME P450, ARACHIDONIC ACID, AND PROTEIN KINASE PATHWAYS**

Cytochrome P450 (CYP₄₅₀) isozymes are a large family of constitutive and inducible heme-containing enzymes which play an important role in the metabolism of xenobiotics.²⁶,²⁷ The P450s are capable of metabolizing a wide variety of carcinogens such as polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines.²⁷,²⁸ Greatest attention, however, has focused on CYP1A1, CYP2A6, and CYP3A4, which are selectively involved in the metabolism of these carcinogens.²⁹ These metabolites are generally activated forms of the pro-carcinogens that subsequently interact with the DNA of target cells. P450s are overexpressed in a variety of human tumors including breast, colon, and lung.³⁰–³² Patterns of tumor-specific P450 expression (such as CYP1B1) have been found in rodent liver tumors.²⁹ The presence of tumor-specific P450 has therapeutic implications that can offer protection against cancer.

Resveratrol has recently been shown to inhibit some CYP₄₅₀ isozymes. Several aromatic hydrocarbons (AH) are known to induce CYP1A1 gene transcription by binding to the Ah receptor, causing translocation to the nucleus, interaction with the promoter of CYP1A1 gene, and up-regulation of CYP1A1 mRNA and protein levels.²⁹ It has been reported that resveratrol inhibits this Ah-induced CYP1A1 expression and activity which is mediated by several AHs such as benzo[a]pyrene (B[a]P), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and dimethylbenz[a]anthracene (DMBA).³³,³⁴ With B[a]P and DMBA, resveratrol appears to inhibit the binding of B[a]P-activated nuclear Ah receptor to the xenobiotic-response element of the CYP1A1 promoter without directly binding to the receptor.³⁴ In contrast, Casper et al.³⁵ have shown that resveratrol serves as an antagonist for the Ah receptor. The compound promotes Ah receptor translocation to the nucleus, but inhibits transactivation of dioxin-responsive genes such as CYP1A1 and ILE. Resveratrol also inhibits other isoforms of CYP₄₅₀ associated with the dealkylation of benzylresorufin, ethoxyresorufin and methoxyresorufin.³⁶ There seems to exist some amount of selectivity with which resveratrol distinguishes and inhibits the activity of CYP1A1 over CYP1A2. Of these two isoforms, CYP1A1 is an extrahepatic enzyme
that is considered relatively important for chemoprevention. Nonetheless, other isoforms of CYP450, such as CYP1B1, described previously to be crucial for tumor progression, are also inhibited by resveratrol. Chang et al. have shown that resveratrol suppresses CYP1B1-catalyzed 7-ethoxyresorufin O-dealkylation activity and mRNA expression without any observed toxicity in MCF-7 cells. Another subtype of CYP450, CYP3A4, predominantly overexpressed in colon and liver cancers, was also shown to be inactivated by resveratrol.

AA is metabolized by the COX pathway to PGs, which mediate several physiological responses. COX exists in two isoforms: constitutive COX-1 is important in maintaining mucosal integrity, gastric microcirculation, and motor functions, and inducible COX-2, which is triggered by cytokines and endotoxins, has been implicated in inflammatory reactions. COX-2 also plays an important role in tumorigenesis as suggested by up-regulation in transformed cells and various forms of cancer. Another pathway by which AA is metabolized is via lipooxygenase (LOX) to produce hydroxyeicosatetraenoic acids (HETEs) or leukotrienes. The exact role of LOX is not known; however, an increased level of this enzyme has been identified in bronchitis, hepatitis, and arthritis. In addition, LOX-derived metabolites have an indirect influence on the development and progression of human cancers.

Early studies have shown that resveratrol, isolated from the roots of Polygonum species, inhibited the activity of rat peritoneal polymorphonuclear 5-LOX and COX products. Subsequent work in our laboratory showed that resveratrol had cancer chemopreventive activity in assays modeling three major stages of carcinogenesis. We first identified resveratrol as chemopreventive agent on the basis of its ability to inhibit the COX and hydroperoxidase activity of COX-1. On the basis of these results, we investigated the anti-inflammatory activity of resveratrol in a rat paw edema model. Resveratrol significantly suppressed both the acute and chronic phases of edema. In addition, resveratrol was shown to suppress the development of preneoplastic lesions in DMBA-treated mouse mammary glands. No signs of toxicity were observed, as judged by morphological examination of the glands. Finally, we studied tumorigenesis in the two-stage mouse skin cancer model in which DMBA was used as initiator and TPA as promoter. 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 98%, respectively, and the percentage of mice with tumors was reduced by 50, 63, 63, or 88%, respectively.

In another study, resveratrol has been shown to inhibit the activity of the COX-1 enzyme derived from sheep seminal vesicles. It was also shown in rats that resveratrol reversed mild water immersion and restraint stress (WRS)-induced gastric protection and blood flow, and attenuated the increase in PGE2 caused by WRS, demonstrating it was a specific COX-1 inhibitor. However, our recent collaborative effort has shown that resveratrol suppresses activation of COX-2 gene expression and activity by interfering with the protein kinase C (PKC) signal transduction pathway in mammary epithelial cells. Resveratrol also inhibited PKC, ERK1, and c-Jun induced COX-2 promoter activity. Further, resveratrol (in addition to other resorcin-type molecules) suppressed basal levels and TGFα-induced COX-2 promoter-dependent transcriptional activity in colon cancer cells. Moreno has
shown that resveratrol inhibits ROS production, phospholipase A$_2$ (PLA$_2$) activity, AA release, and PGE$_2$ synthesis simulated by fetal calf serum (FCS) or platelet-derived growth factor (PDGF) in 3T6 fibroblasts. The COX-2 protein induced by these agents was down-regulated leading to decreased growth and DNA synthesis.\textsuperscript{46} The same observations were extended in murine resident peritoneal macrophages, when it was shown that resveratrol inhibited LPS and PMA-induced formation of ROS, inhibited COX-2 induction, and caused marked reduction of PG synthesis and AA release.\textsuperscript{47} Resveratrol inhibits the peroxidase but not the cyclooxygenase activity of prostaglandin H synthase-2 (PGHS-2), leading to the accumulation of PGG$_2$, which is a toxic endoperoxide.\textsuperscript{48}

Promotion of initiated cells to form a population of transformed, pre-malignant cells is manifested by changes in oncogenes and tumor suppressor genes.\textsuperscript{49} The phorbol ester tumor promoter receptor PKC belongs to an isozyme family of 11 members.\textsuperscript{50} PKC is a well-established regulatory element in the modulation of a variety of cellular processes such as cell signaling and tumor promotion.\textsuperscript{51} Stewart \textit{et al.}\textsuperscript{52} have reported that resveratrol inhibits the PKC-catalyzed phosphorylation of arginine-rich protein substrate in a non-competitive manner. Resveratrol exhibits a broad spectrum of inhibition against a variety of PKC isozymes, such as cPKC, nPKC, and aPKC.\textsuperscript{52} More significantly, this study has attempted to explain differences in the PKC inhibition potency of resveratrol in mammalian cells versus isolated PKC, since the potency of resveratrol depends on the nature of the substrate and cofactors.\textsuperscript{52} In an independent study, it was shown that resveratrol inhibits the activity of PKC when activated by phosphatidylycholine/phosphatidylserine vesicles greater than activation by Triton X-100.\textsuperscript{53} The authors conclude that inhibition of PKC by resveratrol is dependent on membrane effects exerted near the lipid-water interface. Stewart \textit{et al.}\textsuperscript{54} also found that resveratrol exhibits a more distinguished inhibitory effect on the autophosphorylation reactions of protein kinase D (PKD). Gap junctional intracellular communication (GCIC) is important for normal cell growth and suppression can lead to transformation. Many tumor promoters are known to inhibit GCIC, and Nielsen \textit{et al.}\textsuperscript{55} have shown that resveratrol antagonizes TPA-mediated inhibition of GCIC.

**EFFECT ON CELL CYCLE AND APOPTOSIS**

Apoptosis is a normal physiological process wherein cells undergo programmed cell death with considerable morphological and biochemical changes in cellular structures.\textsuperscript{56} Apoptosis is required to maintain a balance between cell proliferation and cell loss. Since misregulation in this balance can lead to malignant transformation, induction of apoptosis in a transformed cell population suppresses the development of cancer.\textsuperscript{57} Various phytochemicals have been shown to induce apoptosis in malignant cells and this pathway provides a promising strategy to protect against cancer.\textsuperscript{58,59} Resveratrol induces apoptosis in HL-60 cells as demonstrated by DNA fragmentation, an increased proportion of subdiploid cell population, and a time-dependent decrease in Bcl-2 expression.\textsuperscript{60} In the same cell line, Clement \textit{et al.}\textsuperscript{61} reported that resveratrol caused a dose-dependent increase in cleavage of caspase substrate poly(ADP-ribose) polymerase (PARP), and caspase inhibitors could block
this effect. Recent evidence emphasizes the importance of up-regulating the CD95-CD95L system for the control of apoptosis and a number of cytotoxic drugs upregulate their expression leading to CD95-mediated signal transduction, activation of caspases, and ultimately, cell death. Up-regulation of the CD95-CD95L system was also shown to be one of the mechanisms of resveratrol-induced cell death in HL-60 cells, as well as T47D breast carcinoma cells.

CD95-independent mechanisms of cell death caused by cytotoxic agents have also been proposed, and doxorubicin-induced apoptosis operates by a CD95-independent pathway. Similarly, resveratrol has been shown to exhibit CD95-independent apoptosis in another monocytic leukemic cell line, THP-1. It was shown that resveratrol did not cause significant changes in the expression of CD95/CD95L or induce clustering of CD95 receptors in THP-1 cells and that neutralization with anti-CD95 or anti-CD95L did not protect from resveratrol-induced apoptosis. Further, it has been observed that resveratrol induced cell death in CEM-C7H2 leukemia cells in a CD95-independent manner, as judged by lack of change in apoptosis in the presence of antibodies to CD95 or CD95L. Moreover, resveratrol effectively induced apoptosis in a CD95-resistant Jurkat cell line.

From a different perspective, apoptosis can be induced by UV-mediated DNA damage. The most important mediator of this effect is the tumor suppressor gene p53, a gene which is mutated in about 50% of tumors, and the lack of expression or function is associated with an increased risk of cancer. It has been shown with JB6 C1 41 cells that resveratrol suppressed cell transformation and induced apoptosis in a p53-dependent manner. Significantly, apoptosis was induced at the same concentration that was required to inhibit cell transformation. Moreover, resveratrol induced apoptosis in cells expressing wild-type p53, but not in p53-deficient cells. Further mechanistic work in this cell line revealed that resveratrol-mediated apoptosis and activation of p53 is mediated via a complex formation between extracellular-signal-regulated protein kinases (ERKs) and p38 kinase. It was also shown that stable expression of negative mutants of ERK2 or p38 kinase or their inhibitors impaired resveratrol-mediated apoptosis in this cell line. To the contrary, in erythroleukemic cells, apoptosis is a result of oxidative stress and is 5-LOX dependent. Programmed cell death is induced in these cells by activation of 5-LOX, and resveratrol was shown to inhibit this effect in dose-dependent manner. In addition, resveratrol inhibited the activity of 15-LOX, COX and peroxidase activity in these cells with IC50 values ranging from 4.5–40 µM.

Bax, together with the anti-apoptotic gene bcl-2, is a transcriptional target for p53. Bax-bax homodimers act as apoptosis inducers while bcl2-bax heterodimers act as a survival signal for cells. It was shown that in a rat colon carcinogenesis model resveratrol induced pro-apoptotic bax expression in colon aberrant cryptic foci (ACF) but not in the surrounding mucosa. In addition, resveratrol treatment suppressed expression of p21 in normal mucosa but not in ACF.

An increasing body of evidence suggests that formaldehyde (HCHO) generators or capturers can play a role in cell proliferation, differentiation, and apoptosis. Szende et al. have shown that several endogenous and exogenous methylated compounds (including resveratrol in its methylated form) are potential formaldehyde generators that can induce apoptosis. Moreover, this group has reported the simultaneous occurrence of resveratrol and HCHO in white and blue grape berries, and the
interaction of these substances may have a role in apoptosis.\textsuperscript{77} Evidence for \textit{in vivo} induction of apoptosis was obtained when it was shown that i.p. administration of resveratrol to rats inoculated with a fast growing hepatoma caused a significant decrease in tumor cell content, an increase in G\textsubscript{2}/M accumulation, and an aneuploid peak.\textsuperscript{78}

Resveratrol has also been shown to affect the growth and tumorigenic potential of several cancer cell lines as evidenced by inhibition of the expression and function of androgen receptor (AR) in LNCaP (prostate cancer) cells.\textsuperscript{79} Resveratrol down-regulated the expression of androgen-induced genes such as \textit{p21}, in addition to mediating several other effects.\textsuperscript{79} In the same cell line, however, it was found that resveratrol neither altered the expression of nor bound to the AR, but mediated anti-androgenic effects, such as decreased intracellular and secreted PSA levels.\textsuperscript{80} In a related study, it was found that resveratrol mediated growth inhibition and apoptosis in LNCaP cells.\textsuperscript{81} The authors extended these observations to some androgen non-responsive cell lines, whereby resveratrol caused growth inhibition and disrupted the G\textsubscript{1}/S phase transition of the cell cycle without causing apoptosis.\textsuperscript{81}

There have been comparisons of the effect of resveratrol on various breast carcinoma cell lines with different metastatic potentials.\textsuperscript{82} Resveratrol caused an accumulation of cells in S-phase with concomitant reduced expression of Rb and increased expression of p53 and bcl-2 proteins.\textsuperscript{82} The compound was most effective against MDA-MB-435 cells, which are highly invasive.\textsuperscript{82} Resveratrol has also been shown to suppress smooth muscle cell proliferation as seen by a G\textsubscript{1}→S block without induction of apoptosis.\textsuperscript{83} In oral squamous cell carcinoma cells, resveratrol caused growth inhibition, both alone and in combination with quercetin and other polyphenolics, as shown by a decrease in DNA synthesis.\textsuperscript{84,85} Resveratrol was found to be more potent against human gingival epithelial cells than other cells of the oral cavity.\textsuperscript{86} In addition, resveratrol caused a decrease in DNA synthesis and irreversible damage to cell proliferation. It is noteworthy that resveratrol did not mediate any antioxidant effects in these cells compared to quercetin and \textit{N}-acetyl-L-cysteine.\textsuperscript{86} Also, resveratrol significantly inhibited the growth, but not the invasion, of highly metastatic B16-BL6 melanoma cells.\textsuperscript{87}

A considerable amount of work has been ongoing with respect to resveratrol and its cell cycle effects. It appears that resveratrol has the greatest effect on the S-phase with consequent effects on S/G\textsubscript{2} transition. In HL-60 cells resveratrol caused an accumulation of cells in the G\textsubscript{1}/S phase as seen by the absence of G\textsubscript{2}/M peaks.\textsuperscript{88} After a 24-h treatment, resveratrol caused a significant increase in the levels of cyclins A and E along with accumulation of \textit{cdc2} in the inactive phosphorylated form.\textsuperscript{88} Similarly, Hsieh \textit{et al}.\textsuperscript{89} noted that resveratrol induced NO synthase in cultured pulmonary epithelial cells with suppression of cell cycle progression through the S and G\textsubscript{2} phases. This was accompanied by a concomitant increase in the expression of p53 and p21 and apoptosis.\textsuperscript{89}

Ulsperger \textit{et al}.\textsuperscript{90} reported that resveratrol desensitized AHTO-7 human osteoblasts to growth stimulation in response to pretreatment with carcinoma cell supernatants. Greatest inhibition was observed with pancreas, breast and renal carcinoma-derived supernatants, whereas colon and prostate had minimal effects.\textsuperscript{90} In another study with MC3T3-E1 osteoblast cells, resveratrol stimulated proliferation and differentiation as indicated by increased DNA synthesis, alkaline phosphatase and
prolyl hydroxylase activity. At lower concentrations, the production of PGE2 was reduced in these cells.

In addition to the above effects on cell proliferation, utilizing a whole cell bioassay system, resveratrol has been shown to be a potent inhibitor of DNA polymerase activity, an important enzyme required for DNA replication. Ribonucleotide reductases are complex enzymes that catalyze the reduction of ribonucleotides into corresponding deoxynucleotides and are important for S-phase DNA synthesis. Resveratrol was recently shown to suppress the activity of this enzyme and DNA synthesis in mammalian tumor cells thus exhibiting an antiproliferative effect.

In a B[a]P plus 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)–induced lung tumorigenesis A/J mice model, dietary resveratrol (500 ppm) had no effect. All chemopreventive agents in this study were administered during the post-initiation period. The authors conclude that since antioxidants (which act as anti-initiation agents) like resveratrol and curcumin were ineffective, the role of oxidative damage in carcinogenesis by BaP plus NNK in the mouse lung tumor model was questionable.

**STUDIES ON NFκB AND IκB**

NFκB is an inducible transcription factor originally identified as a heterodimeric complex consisting of a 50kDa subunit (p50) and a 65kDa subunit (p65). NFκB is strongly linked to inflammatory and immune responses and is associated with oncogenesis in certain models of cancer. A common feature of the regulation of transcription factors belonging to the Rel family is their sequestration in the cytoplasm as inactive complexes with a class of inhibitory molecules known as IκB. Phosphorylation of IκB leads to degradation of the proteosome, allowing NFκB to translocate to the nucleus where it regulates the expression of genes involved in inflammation, cell proliferation, and apoptosis.

The first evidence of resveratrol affecting the transcription factor was derived from the work of Draczynska-Lusiak et al. In their study, it was demonstrated that oxidized low density lipoproteins (LDL) and very LDL treatment activated NFκB binding activity, and resveratrol attenuated the activation of NFκB in PC-12 cells. Resveratrol mediated suppression of NFκB in mouse macrophage RAW 264.7 cells has been controversial. Tsai et al. demonstrated that resveratrol suppressed the activity of LPS-induced inducible form of nitric oxide synthase (iNOS) as seen by a decrease in NO generation in the culture medium. The effect was mediated through down-regulation of iNOS expression at the mRNA and protein levels. Resveratrol, at a concentration of 30µM, also suppressed activation of NFκB by LPS, and inhibited phosphorylation and degradation of IκBα. However, another group report that in RAW 264.7 cells resveratrol indeed decreased LPS-mediated NO release without affecting NFκB activation. It has been shown that resveratrol suppressed TNF-induced NFκB activation (phosphorylation and nuclear translocation) in a variety of cell lines, such as U-937, Jurkat, and HeLa, induced by several agents including TPA, LPS, H2O2, okadaic acid, and ceramide. The suppression of NFκB by resveratrol coincided with the inhibition of AP-1, another transcription factor that is
involved in invasiveness and tumorigenesis. Furthermore, resveratrol inhibited TNF-induced activation of AP-1, MAPK kinase, c-JNK, ROS generation, lipid peroxidation and caspase activation.\textsuperscript{22} Recently, it has been reported that resveratrol is a potent inhibitor of NFκB nuclear translocation and IκB degradation.\textsuperscript{102} In addition, resveratrol effects are mediated through inhibition of IKK, a regulatory key complex that phosphorylates IκB on serines 32 and 36, and blocked the expression of mRNA-encoding monocyte chemoattractant protein-1, a NFκB-regulated gene.\textsuperscript{102}

**ESTROGEN MODULATORY EFFECTS**

Flavonoids and isoflavonoids, like quercetin and genistein, classified as phytoestrogens, have been reported to display both estrogenic and anti-estrogenic effects.\textsuperscript{103} Although genistein has a 1,000-fold lower potency than estradiol (E\textsubscript{2}), its circulating concentrations in individuals consuming a moderate amount of soyfoods is nearly 1,000-fold higher than peak levels of endogenous E\textsubscript{2}.\textsuperscript{104} Population-based studies have also suggested that consumption of a phytoestrogen-rich diet is protective against prostate and bowel cancer, and cardiovascular disease.\textsuperscript{105} Hence, these phytoestrogens may function as cancer chemopreventive agents in human beings.

Resveratrol has been considered as a phytoestrogen based on its structural similarity to diethylstilbesterol. Resveratrol was shown to bind to the estrogen receptor (ER),\textsuperscript{106} and to activate the transcription of estrogen-responsive reporter genes transfected into MCF-7 cells in a dose-dependent manner.\textsuperscript{105} Moreover, resveratrol was reported to function as a superagonist when combined with E\textsubscript{2}, and to increase the expression of estrogen-regulated genes in MCF-7 cells.\textsuperscript{106} However, subsequent studies could not confirm this superagonist activity. For example, with the same cell line, Lu and Serrero\textsuperscript{107} reported that resveratrol showed antiestrogenic activity, as demonstrated by a suppression of progesterone receptor (PR) expression induced by E\textsubscript{2}. In addition, resveratrol down-regulated the basal levels of \textit{TGFα1} and \textit{IGF 1} and up-regulated \textit{TGFβ2} mRNA. In further studies, it was reported that both isomers of resveratrol exhibited superestrogenic activity at moderate concentrations (10 and 25\textmu M), whereas at low concentrations (0.1 and 1\textmu M), antiestrogenic effects were mediated in transfected estrogen response element (ERE)-luciferase reporter experiments.\textsuperscript{108}

In ER-positive PR\textsubscript{1} pituitary cells, resveratrol enhanced prolactin secretion without causing growth stimulation.\textsuperscript{109} Strikingly, the induction of prolactin secretion was blocked by a pure antiestrogen in this study. Further work with MC3T3-E1 osteoblastic cells has shown that resveratrol increased alkaline phosphatase and prolyl hydroxylase activity in these cells, indicating an estrogenic and bone loss preventive effect.\textsuperscript{110} Both activities could be antagonized by tamoxifen, signifying an estrogenic pathway. In U2 osteogenic cancer cells transfected with ER-AF1-luciferase plasmid, resveratrol caused an estrogenic response, whereas in HepG2 liver cells, resveratrol antagonized the action of E\textsubscript{2} in a dose-dependent manner.\textsuperscript{111} Resveratrol has been shown to exhibit a direct antiproliferative effect on human breast epithelial cells that was independent of estrogen receptor status.\textsuperscript{112} Recent studies have demonstrated that resveratrol together with other polyphenolics (when administered as a red wine concentrate) could suppress the proliferation of
ER-positive and -negative mammary cancer cells at picomolar and nanomolar concentrations. In ER-positive cells, such as MCF-7 and T47D, the effect was attributed to both interaction with ER as well as antioxidant effects as seen by decreased formation of ROS. Previous studies have also shown that resveratrol has a direct antiproliferative effect on human breast epithelial cells that is independent of the estrogen receptor status of the cells.

However, in vivo studies with rat models to establish the estrogenic potential of resveratrol have not confirmed suggestions provided by in vitro tests. Various modes of resveratrol administration have been tested. Turner et al. administered resveratrol orally to weanling rats at concentrations ranging from 1–1,000 µg/day. With lower doses, resveratrol did not affect uterine weight, uterine epithelial cell height, cortical bone histomorphometry, or serum cholesterol. However, at the highest dose, resveratrol could antagonize the serum cholesterol lowering activity of E2.

In another study with immature rats, resveratrol was tested by two different routes of administration (oral and s.c.) at concentrations ranging from 0.03–120mg/kg/day, and no effect on uterus weight was found. In a recent study with immature Wistar rats, resveratrol was injected s.c. at three different concentrations (18, 58, and 575 mg/kg). E2 increased uterine weight, enlarged uterine lumen, and induced hypertrophy of epithelial, stromal, and myometrial cells. In contrast, resveratrol mildly decreased uterine weight, and suppressed the expression of ER-α mRNA and protein, and PR mRNA, similar to antiestrogens. The authors concluded that the anti-inflammatory properties of resveratrol suppress the activation of estrogen signalling similar to other anti-inflammatory agents such as indomethacin.

We performed related studies with ovariectomized female Sprague-Dawley rats that were randomized into various groups after one week of quarantine. Resveratrol (3,000mg/kg diet) was administered in the diet and estradiol (50µg/kg body weight) was dissolved in sesame oil and injected subcutaneously for 30 days. The control group received s.c injections of sesame oil only. Vaginal smears were taken every day from all rats to monitor cell morphology. At the end of the study (day 31), the rats were sacrificed, uteri were removed and cleared of intrauterine fluid, and the weights were recorded. As expected, estradiol caused approximately a three-fold increase in uterus weight compared to the vehicle control group. In the absence of estrogen, resveratrol exhibited no estrogenic activity, and in the presence of estrogen, resveratrol demonstrated no antiestrogenic activity. In support of these conclusions, there were no significant differences in uterine weights compared to respective controls (see Figure 1A). Additionally, daily vaginal smears were taken to monitor estrous cytology. Cells were identified as either leukocytes or nucleated (round), or cornified (irregularly shaped, non-nucleated) epithelial cells. A raw score on a scale of 1 through 5 was assigned for cell populations ranging from entirely leukocytes (indicative of a pro-estrous stage) to entirely cornified (indicative of a diestrous stage). The control group exhibited leukocyte abundance whereas estradiol treatment resulted in predominantly cornified epithelial cells within four days. Resveratrol did not cause any significant differences between the scores compared to the respective controls (Fig. 1B).

The only reported study where resveratrol was shown to have estrogenic properties was in stroke-prone spontaneously hypertensive rats. Resveratrol administered in the diet at a concentration of 5mg/kg/day to ovariectomized rats attenuated an increase in systolic blood pressure. It also enhanced endothelin-dependent vascular
FIGURE 1. A. Effect of resveratrol on uterus weight in ovariectomized rats. Seven week-old ovariectomized female Sprague-Dawley rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). All animals were placed on Teklad 4% rat/mouse chow (Harlan Teklad, Madison, WI), and maintained in accord with institutional guidelines. After one week of quarantine, animals were randomized into groups of seven. Animal cages were placed on the rack randomly to avoid variations due to environmental factors such as light and temperature that may result in a pseudo-estrous state. The groups consisted of (1) vehicle (0.1 ml sesame oil, s.c. injection) control; (2) estradiol-17β (50 µg/kg body weight in sesame oil, s.c. injection); (3) resveratrol (3,000 mg/kg diet); (4) resveratrol (3,000 mg/kg diet) plus estradiol-17β (50 µg/kg body weight in sesame oil, s.c. injection). Animals were observed twice daily and weighed twice weekly for the duration of the study (30 days). At the end of the study, the rats were sacrificed by CO2 asphyxiation. The uteri were removed, an incision was made in each uterus to drain intrauterine fluid, they were dried between filter papers, and the dry weights recorded. Estradiol caused approximately a three-fold increase in uterus weight compared to the vehicle control group ($p < 0.01$). In the absence of estradiol, resveratrol (gray bars) exhibited no significant ($p = 0.32$) estrogenic activity, and in the presence of estradiol, resveratrol demonstrated no significant ($p = 0.20$) antiestrogenic activity. B. Effect of resveratrol on vaginal cell morphology. Resveratrol and/or estradiol were administered to ovariectomized female Sprague-Dawley rats as described above. Vaginal smears were taken daily using an eye-dropper containing 0.85% saline, placed on ringed slides on a slide tray, and observed under a light microscope using a 10× eyepiece and 10× objective. (Figure legend continued on opposite age.)
relaxation in response to acetylcholine and prevented ovariectomy-induced decreases in femoral bone strength in a manner similar to estradiol. Significantly, these effects could be partially antagonized with the pure antiestrogen, ICI 182780. Recently, it was demonstrated that resveratrol could bind to both ER-α and -β with comparable affinity, but with 7,000-fold lower affinity than E₂. It was also shown that the type of estrogen modulatory activity of resveratrol depends on the ERE sequence and ER subtype. Resveratrol was shown to have higher transcriptional activity when bound to ER-β than -α. Moreover, resveratrol showed antagonist activity with ER-α, but not with ER-β. Consistent with this report, we have recently observed that resveratrol mediates antiestrogenic effects in endometrial cancer (Ishikawa) cells by a novel mechanism that involves selective down-regulation of ER-α, but not ER-β, manifested as suppression of estrogen-dependent alkaline phosphatase and ERE-luciferase activities, as well as PR and α1-integrin expression.

As more data accumulate on the estrogen modulatory effects of resveratrol, controversy still persists with regard to its ability to serve as a chemopreventive agent in breast cancer. However, certain conclusions can be drawn based on the reports published thus far. Resveratrol indeed exhibits mixed estrogen agonist/antagonist activity with in vitro systems (such as reporter gene assays). However, these data could not be directly extrapolated to an in vivo situation using the classical uterotrophic assay. Rather, resveratrol appears to have a pure antiestrogenic effect at high doses (575 mg/kg body weight). These doses may not be relevant in a chemopreventive setting, but nevertheless provide evidence that endometrial carcinogenicity (as a result of ER-agonism in the uterus) is not likely to be facilitated by resveratrol as with other estrogen receptor modulators such as tamoxifen. The estrogenic effect seen with hypertensive models may have alternate mechanistic pathways compared to the uterotrophic assays, and the pharmacological differences could be explained by several factors, such as selectivity, relative levels of ER-α and -β, and bioavailability in these tissues. Moreover, we have observed that resveratrol exhibits mixed estrogenic/antiestrogenic properties in some ER⁺ mammary cancer cell lines (as seen by differential effects on reporter gene assays as well as natural estrogen-responsive genes such as PR), acts as a pure antiestrogen in other mammary cell lines and, in rodent models, inhibits the formation of carcinogen-induced preneoplastic mammary lesions and tumors. These studies have led us to speculate that resveratrol could function as a novel selective estrogen receptor modulator (SERM).

**FIGURE 1/continued.** Smears were read immediately and cells were identified as either leukocytes or nucleated (round), or cornified (irregularly shaped, non-nucleated) epithelial cells. A raw score on a scale of 1 through 5 was assigned for cell populations ranging from entirely leukocytes (indicative of a pro-estrous stage) to entirely cornified (indicative of a diestrous stage), and plotted against time. Data points represent the mean of raw scores of each group. Control group (○) receiving only sesame oil had mean scores ranging from 1–2. Estradiol treatment (◇) caused a significant (p < 0.01) increase in the score (4.5–5). Resveratrol with (◇) or without (◇) estradiol did not cause any significant (p = 0.7) differences in the scores, compared to the respective controls.
CONCLUSIONS AND OUTLOOK

In this article, we have briefly reviewed early and recent investigations with resveratrol in basic cancer prevention. Resveratrol represents a relatively new class of chemopreventive agent in comparison with retinoids and other diet-derived compounds. In various in vitro and in vivo models, resveratrol has proved to be capable of retarding or preventing steps of carcinogenesis, several of which are summarized in Figure 2. It has become apparent that resveratrol can mediate differential responses with various tissues, organs, and assay models. Thus, some activities have been or remain controversial. Nevertheless, one factor encouraging further work with resveratrol is that it is uniquely found in a soluble form in red wine, and it is virtually absent in most fruits and vegetables that form a major portion of human diet. However, although wine may be considered a predominant bioavailable dietary source, ingestion of grapes or peanuts may be relevant, and dietary supplements are available. The compound also bears a simple chemical structure that is capable of interacting with a variety of receptors and enzymes, and serving as an activator or inhibitor in a number of pathways. Nonetheless, it is noteworthy that no toxicity reports have been published with respect to resveratrol in animals. In our experience, resveratrol has proven to be non-toxic, even at high doses (3,000 mg/kg diet for 120 days in rats). In addition, since resveratrol is an active ingredient of several traditional medicines used for centuries in India, China, and Japan, the general medicinal

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**FIGURE 2.** Schematic representation of the effect of resveratrol on various pathways of carcinogenesis, and cell proliferation. The upward arrow symbol (↑) indicates targets of resveratrol that are either enhanced or up-regulated, the downward arrow symbol (↓) indicates targets that either suppressed or down-regulated. A question mark indicates controversial data.
value and safety of this compound may be suggested. A challenge for the future will be proper extrapolation of data from in vitro experiments or animal studies to the human situation. As exemplified by this article, a considerable amount of time, research, and financial resources have already been invested in the development and characterization of resveratrol. Synthesis and large-scale production have been accomplished, and preclinical toxicity studies are underway. The overall situation is unique since the compound is already consumed by human beings, so certain benefits may currently be realized, irrespective of our knowledge of mechanism. Nonetheless, full potential can only be realized on the basis of clinical trials, and it appears such trials will be performed in the future.

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