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Review

Resveratrol: A review of preclinical studies for human cancer prevention

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Abstract

The search for novel and effective cancer chemopreventive agents has led to the identification of various naturally occurring compounds one of which is resveratrol (*trans*-3,4′,5-trihydroxystilbene), a phytoalexin derived from the skin of grapes and other fruits. Resveratrol is known to have potent anti-inflammatory and antioxidant effects and to inhibit platelet aggregation and the growth of a variety of cancer cells. Its potential chemopreventive and chemotherapeutic activities have been demonstrated in all three stages of carcinogenesis (initiation, promotion, and progression), in both chemically and UVB-induced skin carcinogenesis in mice, as well as in various murine models of human cancers. Evidence from numerous in vitro and in vivo studies has confirmed its ability to modulate various targets and signaling pathways. This review discusses the current preclinical and mechanistic data available and assesses resveratrol's anticancer effects to support its potential as an anticancer agent in human populations.

Keywords: Resveratrol; Human cancer; Skin cancer; Breast cancer; Lung cancer; Gastric; Colorectal cancer; Hepatoma; Neuroblastoma; Pancreatic cancer; Leukemia

Contents

Abbreviations: NFκB, nuclear factor kappa B; NMSC, non-melanoma skin cancer; COX-2, cyclooxygenase-2; ODC, ornithine decarboxylase; DMBA, 9,10-dimethylbenz[*a*]anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; UVB, ultraviolet B; UVA, ultraviolet A; SCC, squamous cell carcinoma; i.m., intramuscular; EGCG, (−)-epigallocatechin gallate; ER, estrogen receptor; TGF-α, transforming growth factor-alpha; PI3K, phosphoinositide-3 kinase; i.p., intraperitoneal; TEBs, terminal end buds; PKC, protein kinase C; MNU, *N*-methyl-*N*-nitrosourea; AOM, azoxymethane; DMH, 1,2-dimethylhydrazine; ACF, aberrant crypt foci; ROS, reactive oxygen species; NDEA, *N*-nitrosodiethylamine; 5-FU, 5-fluorouracil; BaP, benzo[*a*]pyrene; LLC, Lewis lung carcinoma; HUVEC, human umbilical vein endothelial cells; NNK, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone.

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Introduction

There is growing interest in using naturally occurring compounds as potential cancer chemopreventive agents in human populations. In this regard, a significant correlation between dietary intake and many types of cancer has been shown in epidemiological data generated throughout the world, and in animal experiments, many dietary substances have been documented to have anticancer properties. These include green tea catechins, lycopene, soy isoflavones, pomegranate phenolics, selenium, vitamins E and D, curcumin, silibinin, and resveratrol.

Resveratrol, *trans*-3,5,4′-trihydroxy-*trans*-stilbene is a phytoalexin produced by plants, and the skin of red grapes is particularly rich in resveratrol which affects the processes underlying all three stages of carcinogenesis; namely, tumor initiation, promotion and progression. It has also been shown to suppress angiogenesis and metastasis. Extensive data in human cell cultures indicate that resveratrol can modulate multiple pathways involved in cell growth, apoptosis, and inflammation. The anti-carcinogenic effects of resveratrol appear to be closely associated with its antioxidant activity, and it has been shown to inhibit cyclooxygenase, hydroperoxidase, protein kinase C, Bcl-2 phosphorylation, Akt, focal adhesion kinase, NFκB, matrix metalloprotease-9, and cell cycle regulators. These and other *in vitro* and *in vivo* studies provide a rationale in support of the use of resveratrol in human cancer chemoprevention, in a combinatorial approach with either chemotherapeutic drugs or cytotoxic factors for the highly efficient treatment of drug refractory tumor cells (Seve et al., 2005). This review discusses the current preclinical and mechanistic data available to support its potential use in diminishing risk for a variety of human cancers.

Bioavailability

Resveratrol is produced by plants in response to infection by the pathogen *Botrytis cinerea* (Delmas et al., 2006). It is also induced in response to a variety of stress conditions, such as vicissitudes in climate, exposure to ozone, sunlight and heavy metals (Bavaresco, 2003). It has been detected in more than 70 plant species, including grapes, peanuts, berries, and pines. Fresh grape skin contains about 50 to 100 μg of resveratrol per gram wet weight (Baliga et al., 2005), which contributes to a relatively high concentration of resveratrol in red wine and grape juice.

trans-Resveratrol, also referred to as 3,5,4,′-tri-hydroxystilbene, belongs to the stilbene class of polyphenolic compounds. It exists in both *cis* and *trans* isomeric forms. In plants, it mostly exists in glycosylated piceid forms (3-O-B-D-glucosides). Other minor conjugated forms containing 1–2 methyl groups (pterostilbene), a sulfate group (*trans*-resveratrol-3-sulfate) or a fatty acid have also been identified. Glycosylation is known to protect resveratrol from oxidative degradation, and glycosylated resveratrol is more stable and more soluble and readily absorbed in the human gastrointestinal tract (Regev-Shoshani et al., 2003). In humans, following its absorption, it is readily metabolized in the liver by phase-2 drug-metabolizing enzymes to water-soluble *trans*-resveratrol-3-O-glucuronide and *trans*-resveratrol-3-O-sulfate, accounting for its predominant urine excretion (Walle et al., 2004). Compared to resveratrol, which has a plasma half-life of 8–14 min, the metabolites have a plasma half-life of about 9.2 h (Walle et al., 2004). However, the bioavailability and efficacy of these resveratrol metabolites are unknown (Baur and Sinclair, 2006). Compared to other known polyphenols, such as quercetin and catechin, *trans*-resveratrol is well absorbed much more efficiently following oral administration to humans (Soleas et al., 2001).

Numerous studies exist that have utilized a wide range of concentrations of resveratrol, suggesting that its biological effects may vary depending on cell and tissue types. For example, concentrations between 32 nM and 100 μM have been used to study various effects of resveratrol *in vitro*, and 100 ng–1500 mg/kg (body weight) for animal studies (Baur and Sinclair, 2006). Preclinical studies in rats, using HPLC methods, have suggested that intragastric administration of 20 mg/kg *trans*-resveratrol generated peak values of 1.2 μM in plasma (Asensi et al., 2002). In a separate study, male rats treated with 300, 1000, and 3000 mg/kg body weight per day were reported to achieve plasma concentrations of 576, 991, and 2728 ng/ml, respectively, and whereas in females, it was 333, 704, and 1137 ng/ml (Crowell et al., 2004). A plasma concentration of approximately 1.1 μg/ml was determined to be approximately 5 μM (Crowell et al., 2004). A single oral administration of 14C-*trans*-resveratrol to male Balb/c mice showed preferential binding of radio-labeled resveratrol in the stomach, liver, kidney, intestine, bile, and urine, and penetrated the tissues of the liver and kidney (Vitrac et al., 2003). Both the parent compound and the phase-2 metabolites were also detected in these tissues (Vitrac et al., 2003). In humans, 24.6% of the oral dose administered appeared in the urine,

including metabolites (Soleas et al., 2001), whereas after intragastric administration to rodents, only 1.5% of resveratrol reached the plasma compartment (Asensi et al., 2002). This may reflect, in part, polymorphisms related to intestinal absorption and/or rates of metabolism in the liver and intestine among the species.

Skin cancer

Non-melanoma skin cancer

Skin cancer is the most common type of human malignancy. Each year, more than one million new cases of non-melanoma skin cancer (NMSC) are diagnosed in the Unites States alone. Topical resveratrol has been tested for its efficacy against the development of several cutaneous disorders, including skin cancer (Afaq et al., 2002; Baliga and Katiyar, 2006; Bode and Dong, 2000). A single topical application of resveratrol (25 μmol) to SKH-1 hairless mice significantly inhibited ultraviolet B (UVB) (180 mJ/cm^2)-mediated phototoxicity including enhancement of bifold skin thickness and skin edema (Afaq et al., 2003). Skin hyperplasia induced by multiple exposures to UVB radiation (180 mJ/cm^2; 7 exposures on alternate days) was also inhibited when resveratrol was applied topically at a dose of 10 μmol/animal, 30 min prior to each UVB exposure. The anti-proliferative effects of resveratrol were shown to be modulated by cell cycle regulatory proteins (Reagan-Shaw et al., 2004). Resveratrol decreased the expression of cyclins D1 and D2, Cdk 2, 4 and 6, and proliferating cell nuclear antigen (PCNA) whereas p21WAF1/CIP1 was increased. Furthermore, there was inhibited expression of anti-apoptotic proteins, such as survivin, and markers of tumor promotion, cyclooxygenase (COX)-2, and ornithine decarboxylase (ODC) were observed (Aziz et al., 2005).

The chemopreventive effects of resveratrol were assessed, employing UVB-mediated skin tumorigenesis in the SKH-1 hairless mouse model. Topical application of resveratrol either pre- or post-UVB significantly inhibited tumor incidence and delayed the onset of tumorigenesis. Similarly in a standard chemical carcinogenesis mouse tumor protocol using a two-stage, DMBA-initiated and TPA-promoted murine skin cancer model showed a 98% reduction in skin tumors (Jang et al., 1997). Soleas et al. also found a 60% reduction in papillomas with topically applied resveratrol (Soleas et al., 2002), which could be related to its cytotoxic and free radical scavenging activities (Kapadia et al., 2002). Orally administered resveratrol was also shown to inhibit DMBA/croton oil-induced mouse skin papillomas, correlated with prolonging the latent period of tumor occurrence and inhibiting croton oil-induced enhancement of epidermal ODC activities (Fu et al., 2004). *In vitro* data generated in human squamous cell carcinoma cells demonstrate that resveratrol induces G1-phase cell cycle arrest, accompanied by p21WAF1/CIP1 induction, and it decreases the cell cycle regulators, cyclins D1/D2/E and Cdks, hyperphosphorylated pRb proteins, MEK1 > ERK1/2, and AP-1 signaling (Adhami et al., 2001; Ahmad et al., 2001; Kim et al., 2006).

Much of the work regarding skin tumorigenesis has focused on the effects of UVB radiation, whereas much less is known about UVA-induced signaling pathways and their role in tumor promotion. UVA comprises more than 90% of incident solar radiation that reaches the surface of the earth and has been linked to the development of cutaneous squamous cell carcinomas (SCCs) (Bachelor and Bowden, 2004). Seve et al. showed that resveratrol potentiates generation of 8-oxo-7,8-dihydro-2′-deoxyguanosine in UVA-irradiated genomic DNA in immortalized HaCat human keratinocyte cells. In the presence of resveratrol, UVA was shown to significantly enhance the induction of DNA strand breaks and cell death (Seve et al., 2005). These *in vitro* data suggest the differential effects and/or mechanism of resveratrol in the context of the UV spectrum.

Melanoma

Resveratrol can inhibit growth and induce apoptosis in melanoma cell lines (Hsieh et al., 2005; Niles et al., 2003). However, oral resveratrol (20 mg/kg twice per day or in the drinking water at 23 mg/l) did not inhibit the growth of B16M melanoma cells inoculated into the footpads of mice but, interestingly, it decreased the hepatic metastatic invasion of B16M cells inoculated intrasplenically (Asensi et al., 2002). The intraperitoneal administration of resveratrol at the time of i.m. injection of B16-BL6 cells into syngeneic mice resulted in a dose-dependent delay of tumor growth, without toxicity. In this study, resveratrol, was found to be less effective when compared with other polyphenols tested, such as EGCG, apigenin, and quercetin (Caltagirone et al., 2000). In a human melanoma xenograft model, Niles et al. reported that resveratrol did not have a statistically significant effect on melanoma growth, and it might even stimulate tumor growth at higher dose levels (0.006% in food or 100 mg in slow-release pellets). In addition, piceatannol, a major resveratrol metabolite, did not affect the *in vitro* growth of a murine melanoma cell line, but significantly stimulated the number of lung metastases (Niles et al., 2006). The resveratrol content in the skin of these mice, measured 5 min after a bolus of 75 mg/kg introduced, was found to be 21 nmol/g and 4.67 nmol/g in glucuronide-conjugate forms. A measurable amount of resveratrol was found in the tumors, although it was less than the amount found in the skin. The *in vivo* studies appear to show that resveratrol is not an effective chemotherapeutic agent in inhibiting melanoma growth in animals, although more preclinical studies would be needed for confirmation.

Breast cancer

Resveratrol is considered be a phytoestrogen, based on its structural similarity to diethylstilbestrol, a synthetic estrogen. It can bind to both alpha- and beta-estrogen receptors, and activates estrogen receptor-dependent transcription in human breast cancer cells. Despite a number of studies performed using both hormone-sensitive and hormone-resistant breast cancer cells, the estrogen-modulatory effects of resveratrol remain

controversial (Le Corre et al., 2005). In some cell types, such as estrogen receptor (ER)-positive MCF-7 and T47D cells, resveratrol acts as a superagonist, whereas in others, it produces activation equal to or less than that of estradiol (Gehm et al., 1997). In other studies, Clement et al. (1998) reported Fas/Fas ligand-mediated growth inhibition of T47D by resveratrol. Resveratrol (>1 μM) inhibited the growth of MCF-7 cells by antagonizing the growth-stimulatory effect of 17-beta-estradiol (E2) in a dose-dependent manner (Lu and Serrero, 1999), as well as the highly invasive MDA-MB-435 cells (Hsieh et al., 1999). In the absence of E2, resveratrol was observed to carry out mixed estrogen agonist/antagonist activities in some mammary cancer cell lines, but in the presence of E2, resveratrol (1 nM) was shown to function as an anti-estrogen (Bhat et al., 2001). Resveratrol was also shown to inhibit the proliferation of the estrogen-receptor negative human breast carcinoma cell line, MDA-MB-468, by inhibiting the levels of autocrine growth stimulators, transforming growth factor-alpha (TGF-α), PC cell-derived growth factor, and insulin-like growth factor I receptor, and increasing the growth inhibitor TGF-β2 (Serrero and Lu, 2001).

One implication of these varied *in vitro* results is that the chemopreventive effects of resveratrol are likely to be very complex. In fact, in addition to its antioxidant scavenging of free radicals and modulating ER activity (Magee and Rowland, 2004), resveratrol can interfere with an ERα-associated PI3K pathway, following a process that could be independent of the nuclear functions of the ERα (Pozo-Guisado et al., 2004), and acts as an agonist for the cAMP/kinase-A system (El-Mowafy and Alkhalaf, 2003). It also promotes the accumulation of growth inhibitory/pro-apoptotic ceramide (Scarlatti et al., 2003) and induction of quinone reductase (QR, a phase II detoxification enzyme) (Bianco et al., 2005), and induces caspase-independent apoptosis through Bcl-2 downregulation (Pozo-Guisado et al., 2005). It has been shown to suppress Src tyrosine kinase activity (Kotha et al., 2006a), nitric oxide generation, and the NFκB pathway (Bhat and Pezzuto, 2002).

In adult rats, resveratrol inhibited the formation of estrogen-dependent preneoplastic ductal lesions induced by DMBA in these mammary glands (IC50 = 3.2 μM) and reduced MNU-induced mammary tumorigenesis when administered by gavage, suggesting the potential beneficial effects of resveratrol against mammary tumorigenesis (Bhat et al., 2001). Furthermore, dietary resveratrol (10 ppm) in Sprague Dawley rats produced striking reductions in the incidence (45%; $p<0.05$), multiplicity (55%; $p<0.001$), and extended the latent period of tumor induction by DMBA in a mammary carcinogenesis protocol. This was associated with decreased COX-2 and matrix metalloprotease-9 expression and suppression of NFκB activation (Banerjee et al., 2002). Recently, lifetime administration of resveratrol in the diet (1 g/kg) to rats reportedly reduced susceptibility to mammary cancer in DMBA-induced mammary carcinogenesis, which correlated with a significant reduction in proliferative cells and an increase in apoptotic cells in mammary terminal ductal structures, and more differentiated lobular structures (Whitsett et al., 2006).

Resveratrol supplementation (1 mg/l; 4 μg/mouse) delayed spontaneous mammary tumor development and reduced metastasizing capacity in HER-2/new overexpressing transgenic mice, which develop multiple mammary tumors at an early age. This anticancer effect was associated with the downregulation of HER-2/neu (Provinciali et al., 2005). In human breast cancer xenografts, lower tumor growth, decreased angiogenesis, and an increased apoptotic index was seen in MDA-MB-231 tumors in resveratrol-treated (25 mg/kg/day) nude mice (Garvin et al., 2006). In one study, however, no growth inhibitory effect was observed in highly metastatic 4T1 murine mammary cancer cells when resveratrol was administered intraperitoneally (1–5 mg/kg daily for 23 days, starting at the time of tumor inoculation), although the growth of these cells was inhibited in *in vitro* studies (Bove et al., 2002). The differences in these xenograft experiments remain largely unexplained, but it may possibly be due to inadequate dosing.

The available experimental evidence regarding breast cancer risk and consumption of estrogenic chemicals during critical periods of development indicates that the timing and level of exposure to estrogenic chemicals are likely to be important risk factors. A short treatment of pre-pubertal female Sprague–Dawley rats with high-dose resveratrol (100 mg/kg) enhanced MNU-induced mammary carcinogenesis in an estrogen-free environment, as reflected by significant increases in the incidence and multiplicity of mammary tumors (Sato et al., 2003). These data suggest that pre-pubertal resveratrol exposure affects endocrine function. Although the precise mechanism by which resveratrol accelerates the occurrence of mammary cancer in these mice requires further investigation, an increase in the number of TEBs (terminal end buds) and reduction of their differentiation into alveolar buds has been shown to play a critical role in increasing the risk of breast cancer (Russo and Russo, 1987; Sato et al., 2003). It is unclear whether a high dose of resveratrol mediates this process. Although the dose of 100 mg/kg/day of resveratrol represents 5000 times the amount that is consumed by a person drinking one glass of red wine a day (Juan et al., 2002), these studies point to the need for further investigation to determine whether the potential use of resveratrol and/or other dietary consumption of phytoestrogens as a chemopreventive agent for breast cancer is safe in younger, high-risk populations.

Gastric and colorectal cancer

Because the primary etiological determinants for gastric cancer are thought to be exposure to chemical carcinogens and/or chronic infection with *Helicobacter pylori*, and resveratrol was found to be effective in inhibiting the replication of *H. pylori* (Mahady and Pendland, 2000), this provides a reason for the intervention studies using resveratrol for combating gastric cancer (Atten et al., 2005). A number of cell types respond to resveratrol treatment by manifesting cell cycle arrest and apoptosis that, in part, is mediated through nitric oxide formation, which then interferes with endogenously produced reactive oxygen (Holian et al., 2002). The signaling through PKC alpha and delta activity also seems to be affected by

resveratrol treatment (Atten et al., 2005). The intracellular apoptotic signals engaged by resveratrol may be cell-type-dependent, and may be related to differentiation status in various gastric adenocarcinoma cell lines (Riles et al., 2006). In colon cancer cells, resveratrol activates various caspases and triggers apoptosis, which involves the accumulation of the pro-apoptotic proteins Bax and Bak and redistribution of the Fas receptor in membrane rafts (Delmas et al., 2003). Relatively high concentrations also substantially downregulate telomerase activity (Fuggetta et al., 2006).

The *in vivo* efficacy of resveratrol has been tested in two animal models of colorectal cancer, dimethylhydrazine-induced AOM and mutant *Min* mice. AOM-induced tumors share many histopathologic similarities with human tumors, and they often carry mutations in *K-ras* and β-catenin genes but, unlike human tumors, the *Apc* gene (15%) is less frequently mutated. The *Min* mice harbor a mutated *Apc* gene similar to that found in patients with familial adenomatous polyposis, and in many sporadic cancers (Corpet and Pierre, 2003). Administered orally at 200 μg/kg/day in the drinking water, resveratrol significantly reduced the number of AOM-induced aberrant crypt foci (ACF) associated with changes in Bax and p21 expression (Tessitore et al., 2000). *Min* mice receiving resveratrol (0.01% in the drinking water for 7 weeks) showed a 70% reduction in the formation of small intestinal tumors and prevented colon tumor development. Resveratrol treatment led to the downregulation of genes that are directly involved in cell cycle progression or cell proliferation (cyclins D1 and D2, DP-1 transcription factor, and Y-box binding protein) and the upregulation of genes that are involved in the recruitment and activation of immune cells (cytotoxic T lymphocyte Ag-4, leukemia inhibitory factor receptor, and monocyte chemotactic protein 3) and in the inhibition of the carcinogenic process and tumor expansion (tumor susceptibility protein TSG101, transforming growth factor-beta, inhibin-beta A subunit, and desmocollin 2), suggesting the multiplicity of the molecular targets and signaling cascades (Schneider et al., 2001). An inhibitory effect was also observed in the experiments conducted in the xenograft gastric tumor model, which utilized high doses of resveratrol (500–1500 mg/kg), directly injected beside the tumor body 6 times, at an interval of 2 days (Zhou et al., 2005). The synthetic resveratrol analog 3,4,5,4′-tetramethoxystilbene (DMU-212) also inhibited the development of adenomas in the *Apc(Min+)* mouse (Sale et al., 2005). In addition, the efficacy of resveratrol was replicated in other rodent models of colon chemical carcinogenesis. Resveratrol (8 mg/kg body weight, administered every day for 30 weeks) markedly reduced tumor incidence and the occurrence of histological lesions, as well as the size of tumors in 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in Wistar rats (Sengottuvelan et al., 2006). However, the data generated in *Min* mice are conflicting. Although resveratrol administered in the drinking water strongly reduced the formation of colon and small intestinal tumors (Schneider et al., 2001), the doses used in this study when consumed ad libitum in the diet, up to 90 mg/kg for 7 weeks, were not effective (Ziegler et al., 2004). The reason for this discrepancy is not understood (resveratrol in diet vs. in drinking water).

Lung cancer

A lower risk of lung cancer among consumers of wine compared with consumers of other beverages has been observed, which may be partly attributed to the high resveratrol content, particularly in red wine. Resveratrol alters the expression of PAH (polycyclic aromatic hydrocarbons)-metabolizing genes, such as the cytochrome P450 1A1 (*CYP1A1*) and 1B1 (*CYP1B1*), microsomal epoxide hydrolase (*mEH*), and glutathione S-transferase P1 (*GSTP1*) genes, resulting in the altered formation of carcinogenic benzo[*a*]pyrene (BaP) metabolites in human bronchial epithelial cells (Mollerup et al., 2001). It inhibits the expression of CYP1A1 and CYP1B1 and the generation of reactive diol-epoxides that can bind to DNA forming covalent adducts that cause structural alterations with mutations (Berge et al., 2004a). BaP is ubiquitous environmental pollutant and is also present in cigarette smoke. There are numerous carcinogens in cigarette smoke that are likely involved in the pathogenesis of this type of tumor. Interestingly, BaP metabolization requires the induction of cytochrome P450 1A1 (CYP1A1) through the activation of the AhR. Balb-C mice receiving resveratrol (50 mg/kg/week for 5 weeks) were found to have significantly fewer BPDE-DNA adducts (Revel et al., 2003). Doses of 2.5 and 10 mg/kg resveratrol were also found to significantly reduce the tumor volume (42%), tumor weight (44%) and metastatic potential (56%) in mice bearing highly metastatic Lewis lung carcinomas (LLCs), through the inhibition of DNA synthesis and LLC-induced neovascularization and tube formation of HUVEC (Kimura and Okuda, 2001).

However, dietary resveratrol (68 mg/kg) that did not alter *CYP1A1* and *CYP1B1* gene expression showed no effect on B[*a*]P-induced lung tumorigenesis in A/J mice (Berge et al., 2004b). Similarly, no effect on lung tumor multiplicity was shown in A/J mice fed a diet supplemented with resveratrol (500 ppm) from 1 week after B[*a*]P and 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) treatment until termination (Hecht et al., 1999). No resveratrol or resveratrol conjugates were detectable by HPLC in the lung tissue of animals receiving a resveratrol-supplemented diet. It remains to be defined whether resveratrol given in the diet reaches the lung tissue in sufficient concentrations, or in a biologically active form.

Esophageal tumorigenesis

Esophageal cancer is common worldwide, and generally has a poor prognosis because the diagnosis is often delayed. Smoking, including exposure to polyaromatic hydrocarbons like benzo[*a*]pyrene (BaP), is known to be a major risk factor. Resveratrol was shown to suppress the growth of an esophageal cancer cell line EC-9706 (Zhou et al., 2003). The *in vivo* anticancer effects of resveratrol were evaluated at concentrations between 1 and 2 mg/kg body weight in *N*-nitrosomethyl-

benzylamine (NMBA)-induced esophageal tumorigenesis in rats. Resveratrol suppressed both the number and size of NMBA-induced esophageal tumors per rat, by targeting COXs and PGE(2) (Li et al., 2002).

Prostate cancer

The growth inhibitory effect of resveratrol has been demonstrated in various cultured prostate cancer cells, both hormone-sensitive and hormone-refractory, which mimic the initial or advanced stages of prostate carcinoma, respectively. These studies have shown that resveratrol substantially modulates the growth of these cells and alters the expression of more than one set of functionally related molecular targets. Resveratrol can repress different classes of androgen-responsive genes, including prostate-specific antigen (PSA), human glandular kallikrein-2, AR (androgen receptor)-specific coactivator ARA70, and p21WAF1/CIP1 in hormone-responsive cells (Mitchell et al., 1999); activate p53-responsive genes such as *PIG7*, *p300/CBP* and *Apaf-1* (Narayanan et al., 2003); inhibit PI3K/AKT activation; and increase Bax, Bak, Bid, and Bad (Aziz et al., 2006). Resveratrol downregulates PSA by a mechanism independent of changes in AR (Hsieh and Wu, 1999, 2000). No or minimal interaction of resveratrol with AR was detected (Kampa et al., 2000). Moreover, resveratrol was shown to modulate NO production (Kampa et al., 2000) and prevent the increase in reactive oxygen species (ROS) (Sgambato et al., 2001). Resveratrol treatment of various prostate cells also accompanied the activation of MAPK signaling and an increase in cellular p53 content, likely due to stabilization by serine-15 phosphorylation (Gao et al., 2004; Lin et al., 2002), ceramide-associated growth inhibition (Sala et al., 2003), and the blockade of Stat3-mediated dysregulation of growth and survival pathways (Kotha et al., 2006a). Currently no preclinical studies have been reported on prostate carcinogenesis.

Hepatoma

Oxidative stress has been implicated in the pathogenesis of liver cancer and, therefore, the use of antioxidants as a therapeutic or preventive agent has been recommended. *In vitro*, resveratrol was shown to block the ROS-potentiated invasion of the hepatoma cells (Daiki et al., 2004) and induce apoptosis (Michels et al., 2006). The pre-treatment of the mice with resveratrol at a dose of 2.5 mg/kg body weight for 2 weeks was also shown to block *N*-nitrosodiethylamine (NDEA)-induced ODC and COX activities in hepatic tissue (Khanduja et al., 2004). In the transplantable murine hepatoma22 model, resveratrol could induce the S phase arrest of H22 cells after the tumor-bearing mice were treated with 10 mg/kg or 15 mg/kg resveratrol for 10 days (Wu et al., 2004). It also enhanced the anti-tumor effect of 5-FU, suggesting that resveratrol could be a biochemical modulator to enhance the therapeutic effects of 5-FU for hepatocellular carcinoma. Resveratrol caused a significant decrease (25%) in tumor cell content by inducing apoptosis. In this study, it was shown that resveratrol treatment also induces an accumulation of cells in the G2/M phase in rats inoculated with a fast-growing tumor (the Yoshida AH-130 ascites hepatoma) (Carbo et al., 1999). The inhibition of the cell cycle progression was further shown to involve decreases in the expressions of cyclin B1 and p34cdc2 in murine transplantable liver tumors, following the administration of resveratrol at 10 or 15 mg/kg bodyweight for 10 days (Yu et al., 2003). Dietary resveratrol (10 or 50 ppm) is also shown to have anti-tumor growth effects and anti-metastasis effects in Donryu rats subcutaneously implanted with an ascites hepatoma cell line of AH109A (Miura et al., 2003). In another study, daily i.p. injections of resveratrol at 1 mg/kg for 7 days were shown to be effective in inhibiting ascites hepatoma in rats (Carbo et al., 1999).

Neuroblastoma

In neuronal-like cells, such as the human neuroblastoma SH-SY5Y resveratrol was shown to inhibit caspase-7 activation, as well as degradation of poly-(ADP-ribose)-polymerase, which occur in cells exposed to paclitaxel, an anticancer drug (Nicolini et al., 2001). The neuroprotective action of resveratrol was suggested to occur through modulating the signal pathways that commit these neuronal-like cells to apoptosis. Resveratrol was shown to induce S phase arrest, preventing SH-SY5Y from entering mitosis, the phase of the cell cycle in which paclitaxel exerts its activity (Rigolio et al., 2005). Furthermore, phosphorylation of Bcl-2 and JNK/SAPK, which specifically occurs after paclitaxel exposure, was reversed by resveratrol (Nicolini et al., 2003). The long-term treatment of neuroblastoma cells with resveratrol is also reported to enhance the differentiation state of the cells (Melzig and Escher, 2002). In stage 4 MYCN-amplified neuroblastoma cell lines, resveratrol was shown to decrease cell viability and induce cell cycle arrest and apoptosis, which accompanied transiently upregulated p53 expression and nuclear translocation of p53, followed by induction of p21 (WAF-1/CIP-1) and Bax expression (Liontas and Yeger, 2004). In mice, intraperitoneal injections of 40 mg/kg body weight resveratrol daily for 28 days suppressed the growth rate of subcutaneous neuroblastomas, resulting in 70% long-term survival (Chen et al., 2004).

Fibrosarcoma

Resveratrol was shown to inhibit FGF2-induced angiogenesis and significantly inhibited platelet/fibrin clot-promoted human colon and fibrosarcoma tumor growth in the CAM (chick chorioallantoic membrane) tumor model (Mousa et al., 2005). Oral administration of resveratrol (1 mg/kg/day) inhibited the growth of a murine T241 fibrosarcoma implanted in C57Bl6/J mice (Brakenhielm et al., 2001).

Pancreatic cancer

Several *in vitro* studies reported that *trans*-resveratrol enhanced apoptosis in pancreatic cancer cells, which is associated with mitochondrial depolarization and cytochrome

c release followed by caspase-3 activation (Mouria et al., 2002). Resveratrol (100 μM) inhibited the proliferation of human pancreatic cancer cell lines, PANC-1 and AsPC-1, in a concentration- and time-dependent manner, and increased the fraction of sub-G0/G1 cells (Ding and Adrian, 2002). Furthermore, treatment with resveratrol was shown to sensitize MiaPaCa2 cells for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through p53-independent induction of p21 and p21-mediated cell cycle arrest associated with survivin depletion (Fulda and Debatin, 2004). Resveratrol can inhibit Src tyrosine kinase activity and thereby blocks the constitutive signal transducer and activator of transcription 3 (Stat3) protein activation, suggesting that Src-Stat3 signaling is a target for resveratrol (Kotha et al., 2006b). However, resveratrol given in the diet at a concentration of 10 ppm failed to show significant effects on BOP (*N*-nitrosobis (2-oxopropyl)amine) initiation of hamster pancreatic carcinogenesis (Kuroiwa et al., 2006). Additional studies are required for further preclinical evaluation of resveratrol efficacy on pancreatic cancer.

Leukemia

A number of *in vitro* studies have demonstrated the anti-proliferative effects of resveratrol in various leukemic cell lines (U937, HL-60) (Gautam et al., 2000; Surh et al., 1999), inducing apoptosis through the CD95-CD95 pathway (Clement et al., 1998). Resveratrol was also shown to induce apoptosis in B-lineage leukemic cells (acute lymphoblastic leukemias) that are resistant to CD95 signaling, through the CD95-independent, mitochondria/caspase-9-specific pathway (Dorrie et al., 2001). Furthermore, involvement of the Cdc42/apoptosis signal-regulating kinase 1/c-Jun N-terminal kinase/FasL signaling cascade was observed in HL-60 cells treated with resveratrol (Su et al., 2005). Downregulation of the two anti-apoptotic proteins, iNOS and Bcl-2, was observed in leukemic B cells from chronic leukemia treated with resveratrol (Roman et al., 2002). Interleukin 1beta (IL-1β) plays a key role in the proliferation of acute myeloid leukemia (AML) cells, and in the AML cell lines OCIM2 and OCI/AML3, resveratrol was shown to inhibit proliferation, arresting the cells at the S phase. Resveratrol significantly reduced the production of IL-1β and suppressed the IL-1β-induced activation of NFκB (Estrov et al., 2003). Interestingly, resveratrol at low concentrations (4–8 μM) was shown to inhibit apoptosis in human leukemia cells via NADPH oxidase-dependent elevation of intracellular superoxide that blocks mitochondrial hydrogen peroxide production, thus resulting in a cellular environment not susceptible to apoptosis induction (Ahmad et al., 2004). The combined effects of resveratrol have been tested with Ara-C or tiazofurin, both antimetabolites, and showed synergistic growth inhibition and apoptosis induction in HL-60 cells (Horvath et al., 2005). *In vivo*, however, only weak potential anti-leukemic resveratrol activity was suggested at a dose of 80 mg/kg body daily for 60 days in mice implanted with a mouse myeloid leukemia cell line, 32Dp210, despite the strong anti-proliferative and pro-apoptotic activities of resveratrol against these cells *in vitro* (Gao et al., 2002).

Conclusion

The attractiveness of naturally occurring compounds for cancer chemoprevention has escalated in recent years. An ideal chemopreventive/therapeutic agent would restore normal growth control to preneoplastic or cancerous cells by modulating aberrant signaling pathways and/or inducing apoptosis. It should target the multiple biochemical and physiological pathways involved in tumor development, while minimizing toxicity in normal tissues (Manson et al., 2005; Mukhtar and Ahmad, 1999a,b; Yance and Sagar, 2006). Resveratrol has been shown to be an effective chemopreventive agent in multiple murine models of human cancers. It has the capacity to interact with multiple molecular targets and appears to be relatively non-toxic at least at the doses tested in these models. However, much work needs to be done: (1) to improve the bioavailability and pharmacologic properties in the different target tissues, and (2) to better understand its exact mechanisms of action in order to predict its efficacy.

The results of these investigations depended critically not only on the agent evaluated, but also on the target population studied and the specific endpoints evaluated (1999). Several phase-I clinical trials are currently underway to evaluate the pharmacokinetics and safety of resveratrol, utilizing various dosages of resveratrol (50 mg–1000 mg) in HIV seronegative, healthy subjects (the Institute of Human Virology) (Baur and Sinclair, 2006). Clinical studies with appropriate endpoints and surrogate markers for anticancer response could help evaluate resveratrol for larger, more intensive clinical trials. A phase-I clinical trial in colon cancer patients (University of California, Irvine) is intended to examine the effects of resveratrol treatment on colon cancer progression and colonic mucosa in colon cancer patients, as well as its effects in modulating the Wnt signaling pathway. Moreover, given the multiple effects of resveratrol and the relatively low dose of resveratrol obtained from red wine or other dietary sources, its health benefit may lie in synergistic combinations with other agents. Resveratrol is shown to have a synergistic effect *in vitro* with both quercetin and ellagic acid for apoptosis (Baur and Sinclair, 2006). Researchers have started to explore resveratrol treatment in combination with other agents in some preclinical studies. Taken together, these studies are expected to provide data on resveratrol's mechanisms of action and provide a foundation for future prevention trials and therapeutic clinical research, utilizing this interesting agent.

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