

Resveratrol: A Candidate Nutritional Substance for Prostate Cancer Prevention^{1,2}

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ABSTRACT The dietary stilbene resveratrol is a major constituent of a variety of edible plant products, including grapes and peanuts. Resveratrol has been identified as an excellent candidate cancer chemopreventive, based on its safety and efficacy in animal models of carcinogenesis. Resveratrol is a prototype of a plethora of bioactive polyphenols in the food supply that has just begun to be mined for cancer preventive agents. For example, polyphenolic grapeseed fractions were shown recently to potently antagonize chemical carcinogenesis. Taking into consideration that the identification of resveratrol as a cancer preventive agent is largely owed to its high abundance in nature (e.g., it accounts for 5–10% of the grapeskin biomass), it is logical to expect that naturally occurring stilbenes that are superior to resveratrol in their cancer preventive properties await identification. Thus, resveratrol may represent the tip of the iceberg of a broad class of stilbene and related polyphenolic natural products that include safe and highly effective agents for cancer prevention. We hypothesize that resveratrol may be especially suitable as a lead agent for prostate cancer prevention given its ability to: 1) inhibit each stage of multistage carcinogenesis, 2) scavenge incipient populations of androgen-dependent prostate cancer cells through androgen receptor antagonism, and 3) scavenge incipient populations of androgen-independent prostate cancer cells by short-circuiting the epidermal growth factor-receptor (EGFR)-dependent autocrine loops in the cancer cells. *J. Nutr.* 133: 2440S–2443S, 2003.

KEY WORDS: • *resveratrol* • *prostate cancer prevention* • *dietary polyphenols* • *protein kinase C (PKC)* • *epidermal growth factor receptor (EGFR)*

Resveratrol (3,4',5-trihydroxystilbene) (**Fig. 1**) is a natural phytoalexin that is abundantly expressed in a small assortment of plant species as a defensive response against fungal infections and other environmental stressors (1). Keen interest has developed in resveratrol based on its evident value as a cancer preventive and cardioprotective dietary substance (1,2). Resveratrol is present in especially high concentrations in peanuts, mulberry skins and grapeskins, and as a consequence, in red wine (1, 2). Resveratrol accounts for 5–10% of the biomass of grapeskins (2), and resveratrol concentrations measured in a sampling of red wine varieties ranged from 2 to 40 μM (2).

Although the pharmacokinetics of resveratrol are incompletely understood, there is reason for optimism that regular consumption of resveratrol may achieve beneficial effects in

target organs (i.e., cardioprotection and cancer prevention). This is based on the efficient intestinal uptake of resveratrol (oral bioavailability), its rapid appearance in the bloodstream and its accumulation over the short term to significant, if not bioactive, levels by various organs, including the liver, kidneys and heart, despite rapid clearance from the circulation (2–4). Although it is encouraging that exposure of rats to high doses of orally delivered resveratrol over an extended period produced no evidence of toxicity in a recent study (5), the question of whether resveratrol can accumulate to bioactive levels in target organs over a period of continued oral administration remains to be addressed (2). Although this information will be key to evaluating the suitability of resveratrol for prostate cancer prevention, which is the focus of this report, the undesired result of insufficient prostate gland accumulation of the phytochemical could inaugurate a search for stilbene natural products that not only have the prostate cancer preventive action characteristic of resveratrol in the in vitro models, but also have superior pharmacokinetic properties.

In this report, we formulate the hypothesis that resveratrol may be especially suitable as a lead agent for prostate cancer prevention (**Fig. 2**), based on findings in recent reports in the literature and ongoing studies in our laboratory. The hypothesis is predicated on the ability of resveratrol to: 1) inhibit each stage of multistage carcinogenesis, 2) scavenge incipient populations of androgen-dependent prostate cancer cells through androgen receptor antagonism, and 3) scavenge incipient populations of androgen-independent prostate cancer

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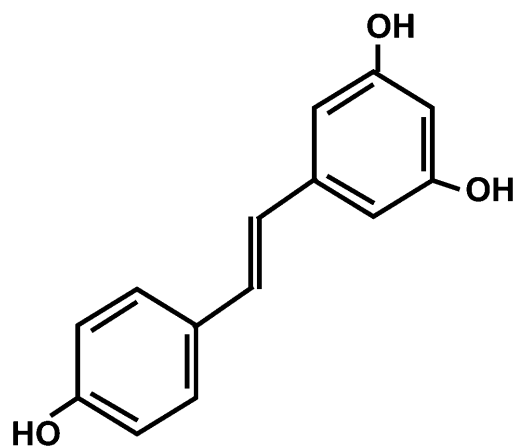


FIGURE 1 Structure of resveratrol (3,4',5-trihydroxystilbene).

cells by short-circuiting epidermal growth factor-receptor (EGFR)⁴-dependent autocrine loops in the cancer cells. The three major lines of evidence in support of the hypothesis, outlined above, are given in-depth consideration under separate headings below.

Inhibition of multistage carcinogenesis by resveratrol

The longstanding dogma of multistage carcinogenesis divides the development of malignancies into the three temporally ordered and mechanistically distinct stages of initiation, promotion and progression (6). Initiation, the first stage, is a rapid mutagenic event that produces a cell with the genetic code for a benign tumor phenotype. The second stage, tumor promotion, is a slow and reversible, epigenetically controlled clonal expansion of the initiated cell that produces a benign tumor (e.g., papillomas in the classical mouse skin model). Tumor progression is the irreversible conversion of the benign tumor to a malignant phenotype (e.g., mouse skin carcinomas) (6). Polycyclic aromatic hydrocarbons (PAHs) are commonly employed to induce initiation and phorbol esters as mediators of tumor promotion and progression stages in mouse skin, the most widely studied carcinogenesis model (6). The seminal report published by John Pezzuto's laboratory in *Science* in 1997 (7) showed that resveratrol has multifactorial effects that check carcinogenesis at each discrete stage and involve interactions between resveratrol and multifarious targets. Resveratrol targets include kinases (8, 9), steroid hormone receptors (10–12) and reactive oxygen species (7,13,14). The *Science* report (7) spawned the now-burgeoning field focused on cancer-preventive effects and therapeutic potential of resveratrol (1, 2). The breadth of this field is reflective of the numerous molecular targets identified for resveratrol in recent years (see below). Sorting out the relative importance of these targets in the cancer preventive activity of the phytochemical is a challenge that must be met in the coming years to move the field forward. In particular, realization of this goal could facilitate identification of more effective cancer preventive agents among stilbene natural products.

Evidence in the Pezzuto report (7) that resveratrol can check the initiation stage of carcinogenesis includes the ability of resveratrol to scavenge free radicals formed in HL60 cells, its

Hypothesis: Resveratrol may prevent prostate cancer by:

- 1) Inhibiting multistage carcinogenesis
- 2) Scavenging incipient populations of androgen-dependent prostate cancer cells

Inhibits androgen receptor function & expression

- 3) Scavenging incipient populations of androgen-independent prostate cancer cells

Short-circuits EGFR-dependent autocrine loops in prostate cancer cells

FIGURE 2 Statement of hypothesis.

antimutagenic activity manifest in a bacterial mutagenesis model and its induction of carcinogen detoxification metabolism in hepatoma cells by induction of the Phase II enzyme quinone reductase. The cellular evidence of free radical scavenging activity of resveratrol (7) is buttressed by rigorous chemical characterization of resveratrol as a free radical scavenger in model systems (14) and other reports. Also apropos of initiation antagonism by resveratrol, a recent report has revealed that resveratrol impedes carcinogen activation by at least two mechanisms (15). Resveratrol inhibits induction of the carcinogen activator cytochrome P-450 1A1 (CYP1A1) by interfering with the binding of the aryl hydrocarbon receptor (AHR) to the promoter of the CYP1A1 gene, and resveratrol directly inhibits CYP1A1 enzymatic activity (15).

Resveratrol potently antagonizes tumor promotion in the DMBA/TPA mouse skin carcinogenesis model (7). The primary target mediating the tumor-promoting activity of the phorbol ester TPA is the protein kinase C (PKC) isozyme family (6). PKC is a family of 10 isozymes, and most are activated by phosphatidylserine (PS)-dependent binding of allosteric cofactors [Ca^{2+} and/or sn-1,2-diacylglycerol (DAG)] to the kinase regulatory domain (16,17). The isozymes are categorized into three subfamilies based on co-factor requirements and structural homology relationships. cPKCs (α , β_1 , β_2 , γ) are Ca^{2+} dependent and DAG responsive, nPKCs (δ , ϵ , θ , η) are Ca^{2+} independent and DAG responsive, and aPKCs (ζ , ι) are independent of Ca^{2+} and DAG (16,17). Phorbol esters activate PKC isozymes through interaction with the DAG binding site (16,17). Although PKD (also referred to as nPKC μ) exhibits the same allosteric co-factor requirements as nPKC isozymes, including responsiveness to phorbol esters, PKD is not a PKC isozyme (16,17). Catalytic-domain homology relationships within the protein kinase superfamily indicate that PKD/nPKC μ is a novel Ser/Thr protein kinase distantly related to calmodulin-dependent protein kinases (17).

We have determined that resveratrol inhibits purified PKC by a catalytic domain-directed mechanism, producing an IC_{50} of 37 μM against the catalytic-domain fragment (CDF) of PKC (8). Kinetic analysis of PKC inhibition indicated that resveratrol competes with the nucleotide substrate ($K_i = 55 \mu\text{M}$) (8). In a comparison of the inhibitory action of resveratrol against a broad spectrum of purified human recombinant PKC isozymes (α , β_1 , γ , δ , ϵ , ζ) and PKD, we found that the PKC-inhibitory mechanism of resveratrol does not involve obstruction of autophosphorylation, which was unaffected by the phytochemical (9). In contrast, resveratrol potently inhibited

⁴ Abbreviations used: AHR, aryl hydrocarbon receptor; AR, androgen receptor; CDF, catalytic-domain fragment; COX, cyclooxygenase; DAG, diacylglycerol; EGFR, epidermal growth factor-receptor; PAH, polycyclic aromatic hydrocarbon; PKC, protein kinase C; PS, phosphatidylserine; ROS, reactive oxygen species.

PKD autophosphorylation (9). These results provide evidence for divergent inhibitory mechanisms against PKC and PKD. In ongoing studies, we have ascertained that resveratrol treatment of cultured human prostate cancer cells provokes differential inhibitory responses among several PKC isozymes and PKD, revealing a selectivity not evident in analyses of the purified kinase species. The PKC isozyme selectivity observed in cells in these studies is concordant with the evident nontoxicity of resveratrol in normal tissues.

As tissue inflammation is provoked by tumor promoters and serves as a driving force in tumor promotion, antiinflammatory agents are viewed as a valuable chemopreventive modality against this stage of carcinogenesis (6). Resveratrol has been shown to exert substantial antiinflammatory activity in an *in vivo* rat model (7). The key molecular targets implicated in the antiinflammatory activity of resveratrol are cyclooxygenases (COX-1 and COX-2). COX-1 and COX-2 are respectively constitutive and inducible enzymes that catalyze the production of proinflammatory prostaglandins from arachidonic acid (6). Resveratrol has global effects against cellular COX activity, through its direct inhibitory action against COX-1 and COX-2 and its suppression of transcriptional COX-2 upregulation (7,18). Because prostaglandins not only stimulate tumor cell growth but also suppress immune surveillance, COX enzymes are likely important targets to the cancer preventive activity of resveratrol (18).

Finally, with regard to resveratrol and tumor progression, resveratrol suppression of Lewis lung carcinoma tumor growth, tumor angiogenesis and metastasis to the lung provides compelling evidence that resveratrol inhibits the final phase of multistage carcinogenesis as well as further elaboration of the malignant phenotype (19). This also is supported by the antiangiogenic action of resveratrol in neovascularization models in normal tissues (20). Also relevant to resveratrol's profile as a candidate cancer preventive agent is its capacity to induce G₁-phase arrest (21) and to trigger mitochondrial-dependent (22), p53-dependent (23) and reactive oxygen species (ROS)-dependent, Bcl-2 sensitive (24) apoptotic responses in tumor cells. It bears noting that the induction of a ROS-dependent apoptotic response by the antioxidant resveratrol, although convincingly demonstrated (24), is counterintuitive. Resveratrol's influence on programmed cell death extends to the flipside of the response, survival signaling. Thus, resveratrol inhibits a key guardian of cell survival, NF κ B, through direct inhibition of I κ B kinase (25).

This overview of molecular targets implicated in the antagonism of tumorigenesis by resveratrol paints in broad brushstrokes the molecular complexity underlying biological responses to the phytochemical. The overview also brings to the fore the enormity of the challenge to sift through resveratrol's targets to identify those with substantial influence over its cancer preventive action, along with the consideration that this may not be possible if the cancer preventive efficacy of this promiscuous phytochemical turns out to depend on concurrent interactions with numerous molecular targets of diverse function. Irrespective of mechanistic complexity, the efficacy of resveratrol against multistage carcinogenesis is an indicator of its potential value as a prophylactic therapeutic for prevention of prostate and other cancers.

Inhibition of androgen-dependent signaling by resveratrol: implications for prostate cancer prevention

Cancer prevention is achieved not only by preclusion, arrest or reversion of premalignant changes that lead to tumor development but also by eradication of incipient populations

of malignant cells. This latter modality of cancer prevention, which includes immune surveillance, nips in the bud nascent malignancies before development into frank neoplastic disease. In addition to the efficacy exhibited by resveratrol in its antagonism of each defined stage of carcinogenesis, resveratrol also harbors the potential to scavenge incipient populations of androgen-dependent prostate cancer cells through its effects on the androgen receptor (AR) (12, 26).

Androgens stimulate proliferation and impede apoptosis of normal prostate epithelial cells and androgen-responsive prostate cancer cells (27). The efficacy of androgen ablation in the treatment of hormone-dependent metastatic prostate cancer is largely accounted for by the induction of apoptosis of neoplastic prostate epithelial cells, with incidental apoptosis of the normal prostate epithelium (28). The neoplastic cells are susceptible to apoptosis upon androgen withdrawal whether dormant or proliferating; this is highly significant in light of the low proliferative index characteristic of neoplastic prostate disease (28).

How do the principles of androgen ablation therapy relate to prostate cancer prevention by resveratrol? Mitchell et al. (12) showed that resveratrol has marked antiandrogenic effects, in the androgen-dependent human prostate cancer cell line LNCaP, that involve repression of the expression of AR, the AR-specific co-activator ARA70 and various AR-regulated genes (e.g., PSA). These effects are associated with cell-growth suppression and induction of apoptosis (12). In a related study, Hsieh & Wu found that resveratrol concentrations sufficient to suppress PSA expression in LNCaP cells did not detectably alter AR expression (26). Together, these studies provide evidence that resveratrol may antagonize androgen action in prostate cancer cells by inhibiting AR activity as well as by suppressing AR expression. These findings offer preliminary evidence that cancer preventive properties of resveratrol may include scavenging of androgen-dependent prostate cancer cells through androgen antagonism. They also suggest the potential value of resveratrol as an adjuvant therapeutic for hormone-dependent prostate cancer.

Inhibition of androgen-independent proliferative signaling in prostate cancer cells by resveratrol: implications for prostate cancer prevention

The unfortunate outcome of androgen ablation therapy of hormone-refractory metastatic prostate cancer is, almost without exception, the emergence of an even more aggressive, hormone-unresponsive form of the malignancy (28). The predictability of this development supports the view that androgen-independent prostate cancer cells are already present in early stages of the disease as "seeds" that "sprout" into frank neoplastic disease in the aftermath of the selective destruction of androgen-dependent prostate cancer cells. Therefore, at least in principle, prostate cancer prevention might entail eradication of incipient populations of androgen-independent prostate cancer cells. One strategy toward this end is to target EGFR-dependent activation of the mitogenic MAP kinases Erk1/2 in androgen-independent prostate cancer cells.

Constitutive Erk1/2 activation is a characteristic feature of androgen-independent neoplastic human prostate epithelial cells, and activated Erk1/2 play a major role in the hormone insensitivity and autonomous growth of the tumors *in vivo* (29–31). Constitutive Erk1/2 activation in the androgen-independent prostate cancer cells is broadly governed by the EGFR (32–35) and can be ablated by EGFR antagonism (36). Ongoing studies in our laboratory are exploring mechanisms by which resveratrol short-circuits EGFR-dependent autocrine

signaling to Erk1/2 activation in androgen-independent prostate cancer cells. These studies may prove germane to the contemplated use of resveratrol as a prostate cancer preventive as well as an adjuvant therapeutic for treatment of advanced disease.

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