

## Molecular Mechanisms of the Chemopreventive Effects of Resveratrol and Its Analogs in Colorectal Cancer: Key Role of Polyamines?<sup>1,2</sup>

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**ABSTRACT** Resveratrol (3,4',5-trihydroxy-*trans*-stilbene), a phytoalexin found in grape skins, peanuts, and red wine, has been reported to exhibit a wide range of biological and pharmacological properties. It has been speculated that dietary resveratrol may act as an antioxidant, promote nitric oxide production, inhibit platelet aggregation, and increase high-density lipoprotein cholesterol and thereby serve as a cardioprotective agent (the so-called "French paradox"). Recently, it was demonstrated that resveratrol can function as a cancer chemopreventive agent, and there has been a great deal of experimental effort directed toward defining this effect. It has been shown that resveratrol and some of its analogues interfere with signal transduction pathways. Thus the activities of various protein kinases are inhibited, the expression of nuclear proto-oncogenes declines, and the activity of ornithine decarboxylase (ODC) is reduced. ODC, which catalyzes the rate-limiting step in the biosynthesis of polyamines, is closely linked with cellular proliferation and carcinogenesis. This review summarizes the recent advances that have provided new insights into the molecular mechanisms underlying the promising properties of resveratrol focusing on the key role of the polyamine metabolism in colorectal cancer cells. *J. Nutr.* 134: 3219–3222, 2004.

**KEY WORDS:** • resveratrol • piceatannol • human cancer cells • polyamines

Colorectal cancer is not only the third most frequent cancer in the world but also one of the most common human malignancies in Western countries. It affects men and women almost equally, with about 400,000 new cases in men and 380,000 in women annually. Almost 400,000 deaths from colorectal cancer still occur worldwide every year. Conventional chemotherapy has no consistent benefit in overall survival. However, in recent years, multidisciplinary research in

epidemiology, molecular biology, and laboratory animal model studies contributed much to the understanding of the etiology of colorectal cancer. More important, these studies enabled the design of highly promising preventive strategies, which are about to influence both the incidence and the prognosis in patients with a high risk of developing this disease (1).

Equally promising, however, are the preliminary data suggesting that various nutrients may act as chemopreventive agents as well (2). Indeed, a wide array of phenolic substances, particularly those present in dietary and medicinal plants, have been reported to possess substantial anti-carcinogenic activities.

Recently, we and others (3–7) reported that the plant polyphenol resveratrol and its analogs have a potent chemopreventive effect in multiple carcinogenesis models both in vivo and in vitro. Resveratrol (3,4',5-trihydroxystilbene, molecular weight = 228.2) is a polyphenol that has been classified as a phytoalexin, because it is synthesized in spermatophytes in response to certain types of stress. It is the active ingredient of the dried roots of *Polygonum cuspidatum*, which has been known in traditional Asian medicine under the name *Ko-jo-kon* (8,9). Resveratrol-containing foods include grapes (10,11), wine (12), and peanuts (13,14). In grapes, especially when infected with *Botrytis cinerea*, resveratrol is exclusively synthesized in the grape skins, which contain 50–100 mg resveratrol/g when they are fresh. Because the grape skins are not fermented in the production process of white wines, only red wines contain considerable amounts of resveratrol. It has been proposed that resveratrol is at least in part responsible for the beneficial effects of a moderate red wine consumption on the development of cardiovascular diseases. Resveratrol inhibited platelet aggregation (15), protected porcine low-density lipoproteins against polyunsaturated fatty acid peroxidation (16), and exerted vasorelaxing effects on endothelium-intact aorta rings of rats (17).

Additionally, the inhibitory potency of resveratrol in various stages of tumor development has attracted much attention (3). This review will summarize our work on the mechanisms and activity of resveratrol and its derivative, focusing on polyamine metabolism as a possible target.

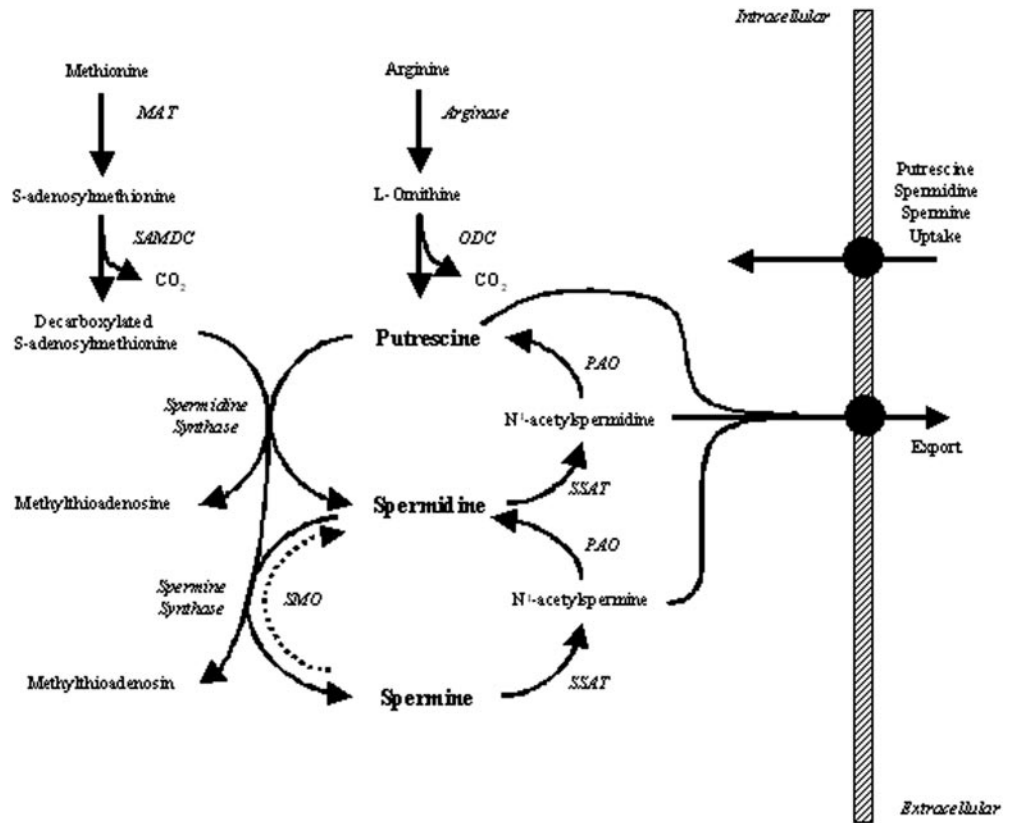
**Polyamines and Colorectal Tumorigenesis.** The naturally occurring polyamines putrescine, spermidine, and spermine are widespread in nature and they have been detected in all eukaryotic cells studied. Tissue polyamine levels are increased either by biosynthesis (generally from ornithine and methionine) or by uptake from extracellular fluids. Conversely, excess cellular polyamines are removed by catabolic reactions converting spermine to spermidine via a  $N^1$ -acetylspermine intermediate and eventually to putrescine via a  $N^1$ -acetylspermidine intermediate. The acetyl polyamines, along with excess putrescine and spermidine, can be excreted to the extracellular fluid. Cellular polyamine homeostasis is maintained through the concerted effort of feedback systems controlling polyamine transport as well as the 3 key enzymes in polyamine metabolism, namely the production of putrescine by ornithine decar-

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**FIGURE 1** Regulation of intracellular polyamine content in mammalian cells [adapted from Ref. (51)].

boxylase (ODC),<sup>4</sup> the synthesis of decarboxylated S-adenosyl-L-methionine decarboxylase (SAMDC, AdoMet-DC), and the acetylation of spermidine and spermine by spermidine/spermine N<sup>1</sup>-acetyltransferase (SSAT) (Fig. 1).

In colon cancer tissue the activities of polyamine-synthesizing enzymes and polyamine content are increased 10- to 15-fold in comparison to normal colonic epithelium (18,19), and polyamines have therefore been considered even as specific markers for neoplastic proliferation in the colon (20). ODC activity and expression have been among the first biomarkers of neoplastic proliferation: as early as 1984 it was shown that ODC activity correlates with risk for neoplastic transformation in patients with colonic adenomatous polyposis (21). ODC activity correlates also with degree of dysplasia in Barrett's esophagus (22) and different stages of colonic carcinogenesis (23). ODC activity is essential for cell proliferation and is required for progression into the S phase of the cell cycle (24). Studies suggest that ODC can be defined as a proto-oncogene (25) and overproduction of this enzyme results in malignant transformation (25–27). More important, however, is that polyamine uptake is upregulated by various mitogens and hormones, as well as by some known tumor promoters (28–31). On the other hand, it has been demonstrated that polyamines stimulated the transcription of *c-myc* and *c-fos* (32).

**Involvement of Ornithine Decarboxylase in Cancer Prevention and Therapy by Resveratrol and Its Analogs.** As stated above, polyamines and their biosynthetic

enzyme ODC are intimately involved in carcinogenesis and malignant growth. It is possible that the anticancer and the chemopreventive activities of resveratrol and its analogs could also be explained by inhibiting ODC/SAMDC and/or increasing SSAT activity. To test this assumption, we and others investigated the effect of resveratrol on polyamine metabolism. TPA-induced ODC activity in mouse 308 cells was inhibited by 48% when treated with 10  $\mu\text{mol/L}$  piceatannol, whereas resveratrol had no inhibitory effect at this concentration (33). Schneider et al. (34) incubated Caco-2 colorectal adenocarcinoma cells with 25  $\mu\text{mol/L}$  resveratrol and detected decreased ODC activity (58% of the control value) after 24 h. The inhibitory effect intensified after 48 h. A direct enzyme inhibition of resveratrol on ODC activity was excluded. We could demonstrate that the decreased ODC activity in Caco-2 cells was accompanied by decreased levels of ODC protein, implicating either increased degradation by the 26S proteasome pathway or impaired ODC synthesis. When mRNA levels of the *odc* gene were determined, a reduction of the *odc* mRNA became evident. *c-Myc* protein was quantified because it controls the *odc* promoter. *c-Myc* was diminished by resveratrol treatment, demonstrating that decreased expression of the *odc* gene is responsible for the inhibition of ODC activity. Piceatannol also exerts an inhibitory effect on ODC activity, with decreased ODC and *c-myc* protein levels and with decreased *odc* mRNA levels, although the inhibitory effect of resveratrol was more potent (7). Whereas Schneider et al. (34) did not observe significant inhibition of SAMDC activity after treatment of Caco-2 cells with 30  $\mu\text{mol/L}$  resveratrol (44), we demonstrated SAMDC inhibition when higher concentrations ( $\geq 50$   $\mu\text{mol/L}$ ) were used. SAMDC was also inhibited by piceatannol, but the inhibitory effect was less pronounced (7).

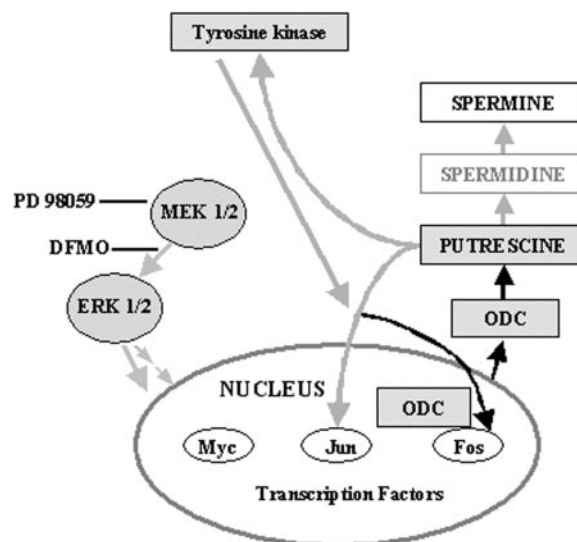
<sup>4</sup> Abbreviations used: DFMO, difluoromethylornithine; MAPK, mitogen activated protein kinase; ODC, ornithine decarboxylase; PAO, polyamine oxidase; SAMDC, S-adenosyl-L-methionine-decarboxylase; SSAT, spermidine/spermine-N<sup>1</sup>-acetyltransferase.

### Polyamine-Catabolizing Enzymes [SSAT and Polyamine Oxidase (PAO)] as Molecular Targets for Resveratrol and Its Analogs.

An early response of human tumor cells to etoposide-induced apoptosis was an increase in PAO activity (35), and increases in SSAT activity have been linked to cytotoxicity in neoplastic cells (36). Cancer cells exposed to polyamine analogs/inducers of SSAT arrest their growth in G<sub>1</sub> and induce the p53-p21<sup>WAF1/CIP1</sup>-Rb pathway, ultimately undergoing apoptosis (37). The induction of both SSAT and PAO can produce an efficient system to generate locally high concentrations of hydrogen peroxide that could effectively induce a signaling pathway ultimately leading to cell death. Both SSAT and PAO activities were found to be decreased in human solid tumors (38). In this study both enzyme activities correlated with prognosis—PAO in a negative manner and SSAT in a positive manner—supporting the idea that polyamine catabolism, particularly oxidation, is linked to tumor growth potential. All these facts, although intriguing, have been studied only partially so far.

Resveratrol and to a lesser extent piceatannol potently upregulated SSAT activity, indicating that these hydroxystilbenes induce polyamine degradation. After 24 h of treatment with resveratrol we observed increased intracellular putrescine and N<sup>1</sup>-acetylspermidine concentrations, whereas the levels of spermine and spermidine did not change significantly (7). Schneider et al. (34) detected reduced levels of putrescine and spermidine after 48 h of treatment with 25 μmol/L resveratrol. The resveratrol analogue *cis*-3,5,4'-trimethoxystilbene decreased ODC and SAMDC activities at a concentration of 0.3 μmol/L with a concomitant reduction of putrescine concentrations after 24 h (39). *c*-Fos and *c*-jun are part of the transcription factor complex AP-1, which can consist of different Fos or Jun family proteins. The individual combination of these proteins seems to be responsible for the effect of the transcription factor. Proliferation, differentiation, or apoptosis can be the result of enhanced AP-1 binding activity. *c*-Fos is implicated in the process of differentiation and programmed cell death. Resveratrol treatment led to increased levels of *c*-fos and *c*-Jun in Caco-2 cells. Only the DNA-binding activity of *c*-fos increased, whereas *c*-jun binding activity remained unchanged (7). These results may be mediated by the increased putrescine levels measured after 24 h, which were caused by enhanced SSAT activity. Accumulation of intracellular putrescine concentration induces *c*-fos (40).

It has been well established that growth factors (mitogens) bind to specific receptors located on the cellular membrane. The mitogen-receptor complexes then trigger a cascade of events including the activation of Ras, which activates the kinase Raf and suppresses expression of SSAT by inhibition of peroxisome proliferator-activated receptor γ (41). The activation of protein kinases is regarded to be the next step in signal transduction. Mitogen activated protein kinases (MAPKs) are phosphorylated by MAPK/extracellular signal regulated kinases, which are, in turn, activated by Raf. MAPKs next trigger the expression of the nuclear oncogenes *myc*, *jun*, and *fos* (Fig. 2), which function as transcription factors, stimulating proliferation and the expression of the ODC gene (42,43). Polyamines, which are formed by ODC, enhance the expression of protein kinases (44) and nuclear oncogenes (32). Difluoromethylornithine (DFMO), which inhibits polyamine synthesis, prevents the expression of protein kinases (44) and nuclear oncogenes (32); resveratrol also inhibited MAPK activity (45). The inhibitory effect of resveratrol on polyamine metabolism in carcinoma cells is presumably mediated by a different pathway. Diminished ODC activity is very likely



**FIGURE 2** Effects of putrescine on signal transduction pathways involved in cell proliferation. The diamine putrescine, which is formed from ornithine by ODC, stimulates tyrosine kinases and the expression of the nuclear protooncogenes *c*-fos and *c*-jun [for extended review see (52)].

mediated by decreased *c*-myc protein levels as demonstrated in Caco-2 cells, because *c*-myc regulates *odc* expression (7). *c*-Myc expression is regulated by the transcription factor E2F1. We and others have demonstrated that resveratrol treatment leads to decreased retinoblastoma protein phosphorylation and thus inactivation of E2F family members (4,46). These data suggest that ODC inhibition could be a result of the cell cycle inhibitory effect of resveratrol. Most studies concerning polyamine metabolism as a target for chemoprevention or cancer treatment have focused on inhibition of ODC. This approach has not proved efficient under in vivo conditions, because ODC inhibition led to an increased polyamine uptake from food, which neutralized cytostatic effects of DFMO (27,47). Results obtained from studies with novel polyamine analogs have suggested that induction of SSAT is a more promising approach to chemoprevention (48–50).

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