DOI 10.1002/mnfr.200400094 Mol. Nutr. Food Res. 2005, *49*, 462 – 471

Review

Resveratrol and breast cancer chemoprevention: Molecular mechanisms

Ludovic Le Corre, N. Chalabi, L. Delort, Yves-Jean Bignon and D. J. Bernard-Gallon

Laboratoire d'Oncologie Moléculaire, CBRV, Centre Jean Perrin, Clermont-Ferrand, France

Despite years of intensive research, breast cancer remains a major cause of death among women. New strategies to combat breast cancer are being developed, one of the most exciting of which is the use of chemopreventive agents. Resveratrol (RES) is a polyphenolic compound found in plants that seems to have a wide spectrum of biological activity. RES has been shown to afford protection against several types of cancer. This review summarizes the chemopreventive effects of RES at the three major stages of breast carcinogenesis: initiation, promotion, and progression. It has anti-oxidant and anti-inflammatory properties, and may induce apoptosis as well as modulate cell cycle and estrogen receptor function in breast cancer cell lines. Although RES has shown remarkable promise as a potent chemopreventive agent in breast cancer, further studies are needed to etablish its usefulness.

Keywords: Breast cancer / Chemoprevention agent / Phytoestrogen / Resveratrol / Review

Received: November 29, 2004; revised: February 3, 2004; accepted: February 11, 2004

Contents

1 Introduction

Breast cancer is a major cause of death in women. It is the third most frequent cancer overall, throughout the world. Genetic and environmental factors play a significant role in breast cancer development with family history being an important factor for determining breast cancer risk. In fact, cancer development results from interaction between genetic factors, environment, and dietary factors which modulate carcinogenesis. A large body of epidemiologic data supports the fact that diet and nutrition play a vital role in carcinogenesis. Global differences in breast cancer incidence and studies of migrating populations suggest that environmental and/or dietary factors play a significant role [1]. The highest reported rates of breast cancer incidence are for white or Hawaiian women in the United States, and significantly lower rates are observed for women in Africa and Asia [2]. Second- and third-generation female descendants of migrants from low-risk to high-risk regions experience rates of breast cancer incidence approaching those of the host country [2], strongly implicating lifestyle factors as the major contributors to the development of the disease [3]. Epidemiological studies performed during the last 20 years support an inverse relationship between diet and the development of cancer. The intake of fruits and vegetables is associated with decreased risk of cancer.

Resveratrol (RES) (3,5,4′-trihydroxystilbene) was first discovered by Michio Takaoka more than 60 years ago, in the resin of *Veratum grandiflorum* (false hellebore) [4]. Then, RES was also detected in grapevines (*Vitis vinifera)* in 1977 by Langcake, who found that the compound was synthesized by leaf tissues in response to fungal infection or exposure to ultraviolet light. This property classifies it as a phytoalexin, compounds produced by plants in response to

Correspondence: Dr. Yves-Jean Bignon, Laboratoire d'Oncologie Moléculaire, Centre Jean Perrin, 58 rue Montalembert, B.P. 392, F-63011 Clermont-Ferrand Cedex 1, France
E-mail: Yves-Jean.Bignon@cjp.fr
Fax: +33-4-7327-80 42

Abbreviations: ER, estrogen receptor; **RES**, resveratrol

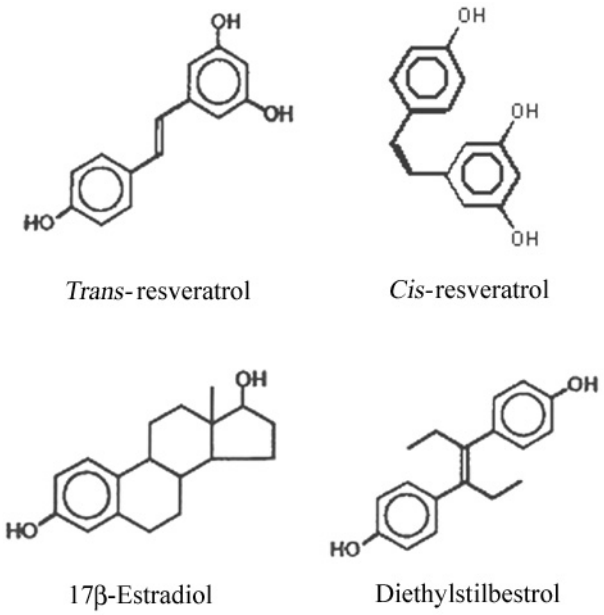

Figure 1. Chemical structure of *cis*- and *trans*-resveratrol, diethylstilbestrol (synthetic estrogen), and 17ß-estradiol.

damage. Consequently, RES levels fluctuate in plants following exposure [5]. RES is a polyphenol and a member of the stilbene family. It can be found in the *cis*- or *trans*-configuration and in a glycosylated form [5]. It has been found in many plants, including grapes, peanuts, berries, *Polygonum* roots, and traditional oriental medicine plants [6]. RES has been reported to be a phytoestrogen due to its structural similarity to the estrogenic agent diethylstilbestrol (Fig. 1). In recent years, research on RES has described several beneficial effects of this compound to human health. It has been reported to have both anticarcinogenic and cardioprotective activities, which could be attributed to its antioxidant and anticoagulant properties [7]. RES has been reported to be effective in inhibiting platelet aggregation and lipid peroxidation, altering eicosanoid synthesis, modulating lipoprotein metabolism [8–10], and exhibiting vasorelaxing and anti-inflammatory activities [11, 12]. For its anticarcinogenic activities, potential mechanisms of RES action have been studied extensively, though there is no clear consensus on the matter. RES has been reported to inhibit the three major stages of carcinogenesis: initiation, promotion, and progression [13]. Anti-initiation activity was indicated by antioxidant and antimutagenic effects and induction of phase II drug-metabolism enzymes. Antipromotion activity was indicated by anti-inflammatory effects and inhibition *in vitro* of cyclooxygenase and hydroperoxidase. Antiprogression activity was described as an induction of human promyelocytic leukemia cell differentiation. RES also inhibits the development of preneoplastic lesion in carcinogen-treated mouse mammary glands in culture and inhibits tumorogenesis in a mouse skin cancer model [13]. The aim of this review is to summarize the molecular mechanistic basis for the potent chemopreventive effect of RES in breast cancer.

2 Resveratrol and breast cancer chemoprevention

Many *in vitro* studies have addressed the RES activities in breast cancer cells. RES exhibits an action in both hormone-sensitive and hormone-resistant breast cancer cells. RES has also been reported to exhibit anti-initiation, antipromotion, and antiprogression activities in breast cancer cells, where these properties seem to be related to regulation of xenobiotic carcinogen metabolism and anti-inflammatory, antiproliferative, and proapoptotic effects.

2.1 Resveratrol: a phytoestrogen

The phytoestrogenic character of RES was confirmed by its capacity to bind and activate α- and β-estrogen receptors (ERs) regulating transcription of estrogen-responsive target genes. However, although a number of studies have been conducted, the effects of RES on ERs remain controversial. For example, with MCF-7 cells in culture, Gehm *et al.* [14] showed that RES (3 – 10 μM) is a superagonist when combined with E2, while Lu and Serrero [15] reported ER antagonism of RES (5 μM) in the presence of E2 and partial agonism in its absence [15]. Bowers *et al.* [16] observed partial to full agonism in CHO-K1 cells transfected with ERα or ERβ and reporter genes based on various estrogen receptor element (EREs). The authors showed that RES (100 μM) acts as a mixed agonist/antagonist in cells transiently transfected with ER, and mediates higher transcriptional activity when bound to ERβ than to ERα. Moreover, RES showed antagonist activity with ERα, but not with ERβ [16]. These data were confirmed with endometrial cancer cells [17]. Based on these reports, it appears that the ability of RES to act as an ER agonist varies between different cell types and dosage. The degree of agonism seems to depend upon the promoter context of the ERE [18]. In the same report, it was reported that RES estrogenicity (5 – 50 μM) also depends on the AF-1 and AF-2 domains of ERα. The role of the AF-1 and AF-2 domains in ERα-mediated gene transcription varies depending on both the activating ligand and the target gene [18].

2.2 Xenobiotic metabolism

Many epidemiological studies have described the effects of exposition to chemical agents in cancerogenesis. The majority of these molecules acquire carcinogenic potential after biotransformation catalyzed by phase I and II detoxi-

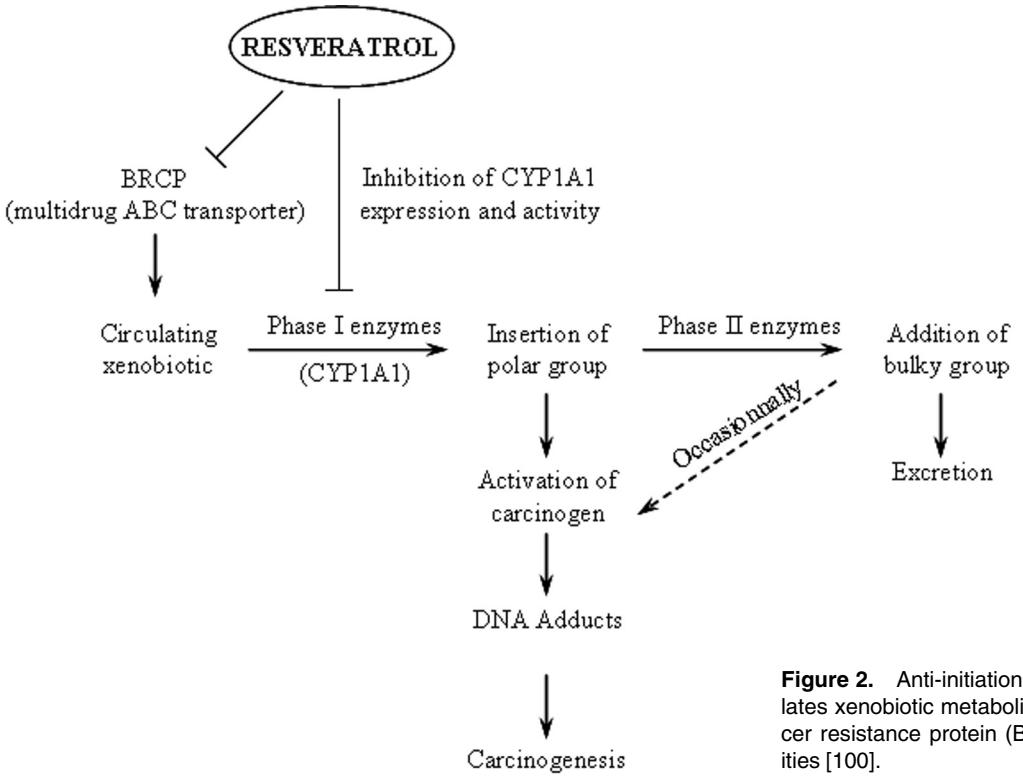

Figure 2. Anti-initiation effects: resveratrol modulates xenobiotic metabolism by altering breast cancer resistance protein (BRCP) and CYP1A1 activities [100].

fying enzymes. Most chemical carcinogens are genotoxic, causing DNA damage by forming covalent adducts with DNA nucleotides. One of the most important phase I detoxifying enzymes involved in the formation of carcinogens is the cytochrome P450 isoenzyme 1A1 (CYP1A1). CYP1A1 is induced by polycyclic aromatic hydrocarbons (PAHs) and xenoestrogens, such as polychlorinated biphenyls (PCBs) which are stored in breast adipose tissue and may play a role in breast oncogenesis [19–21]. PAHs have been suggested to play a causative role in breast cancer etiology [19, 22]. Several PAHs are metabolized to mutagenic and/or carcinogenic metabolites by CYP1A1 [23, 24]. The transcriptional induction *CYP1A1* is under the control the Ah receptor [25, 26]. The binding of PAH ligands to the AhR results in increased expression of CYP1A1, CYP1A2, CYP1B1, and phase II enzymes, which are responsible for conjugation/detoxification of the reactive metabolites. RES was reported to alter the expression of cytochrome P450 and appeared to be a selective inhibitor of human CYP1A1 [27]. RES (1–10 μM) also was shown to inhibit DMBA-induced CYP1A1 enzyme activity and *CYP1A1* transcription in the breast cancer cell line MCF-7 [28]. In T47D breast cancer cells, inhibition of *CYP1A1* transcription by RES (10–100 μM) was attributed to an increase in *CYP1A1* mRNA degradation and to an AhR-independent post-transcriptional pathway [29]. To date, no studies have investigated the effects of RES on other phase I detoxifying enzymes in breast cancer cells.

For phase II detoxifying enzymes, studies have focused the effects of RES on their activities or expression levels. After phase I hydroxylation, xenobiotics are conjugated into inactive compounds amenable to metabolic elimination. Such phase II enzymes include glutathione-*S*-transferase, *N*-acetyl-transferase, uridyl-diphosphoglucuronosyl-transferase, and menadione oxido-reductase and RES failed to modulate their expression. Other enzyme systems, such as *O*-acetyl-transferase and sulfotransferase, collectively referred to as phase II carcinogen activators [30–32], are inhibited by RES (50 μM) in human breast cancer cell lines (MCF-7 and ZR-75-1) and in the cytosol of mammary tumor tissue [32].

RES was reported to decrease the activity of breast cancer resistance protein (BCRP) by modulating both its transport function and ATPase activity in the breast cancer cell line MCF-7. BCRP is a recently discovered multidrug ABC transporter and is a member of the ABCG 'half-transporter' subfamily [33]. It is located at barrier sites [34] where it influences entry of xenobiotic material. Thus, RES-mediated inhibition of BRCP activity may decrease the entry of ingested material to the circulation, affecting drug accumulation and cell viability following cytotoxic drug exposure. These data suggest that RES may have preventive effects on the production of carcinogenic compounds and the formation of DNA adducts (Fig. 2).

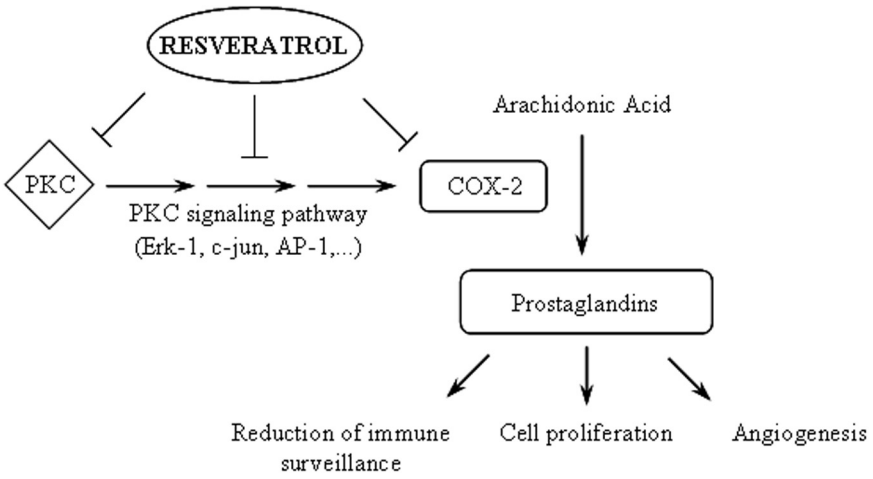

Figure 3. Anti-inflammatory effects: resveratrol decreases the production of prostaglandins by inhibiting COX-2 expression and activity at multiple levels.

2.3 Anti-inflammatory activities

Inflammatory mediators, such as prostaglandins (PGs) and nitric oxide (NO), stimulate the growth and spread of tumors by inducing cellular proliferation [35], reducting immune surveillance [36], and promoting angiogenesis [37]. The enzymes COX-1 and COX-2 and the nitric oxide synthase (NOS) enzymes are responsible for the production of these mediators [38]. *In vitro* studies in non-breast inflammatory models have shown that RES (1–10 μM) significantly decreased NO production [39] and that RES (15 μM) inhibited cyclooxygenase and the hydroperoxidase activities of COX-1 and COX-2 [13]. In human mammary epithelial cells, RES (2.5–20 μM) suppressed phorbol ester (PMA)-mediated induction of PG synthesis by inhibiting *COX-2* gene expression and the activity of COX-2. RES suppressed PMA-mediated activation of *COX-2* transcription by inhibiting the PKC signal transduction pathway at multiple levels. It blocked both PMA-induced translocation of PKC activity from the cytosol to the membrane, the induction of *COX-2* promoter activity by ERK-1 and c-jun and it supressed PMA-mediated induction of *c-jun* and AP-1 activity [40]. On the other hand, the importance of the transcription factor NF-κB in promoting tumorigenesis has been well recognized. Activation of the NF-κB/Rel transcription family, by nuclear translocation of cytoplasmic complexes, plays a central role in inflammation through its ability to induce transcription of proinflammatory genes [41]. NF-κB regulates the expression of a multitude of critical genes including immunoreceptors, transcription factor-associated proteins (c-myc and p53), cell adhesion molecules and enzymes involved in tumor metastasis (COX-2, inducible nitric oxide synthase, and MMP-9) [42–44]. RES was found to alter NF-κB expression and activity in multiple cancer cell line [45]. RES was found to suppress the NF-κB activation in MCF-7 breast cell lines [46]. Thus, NF-κB is described as a potential target in chemoprevention of RES [45]. Thus, expanding body of evidence suggests that RES acts as a tumor antipromotion agent and that inhibitors of COX-2 are useful for treating inflammation and preventing cancer [47–49] (Fig. 3).

2.4 Antiprogression activities

The loss of cell cycle control and lack of normal apoptosis are two of the hallmarks of malignant cells. Many studies have shown that RES suppresses growth of breast cancer cell lines and inhibits the activity and expression of several enzymes that play key functions in the regulation of the cell growth and apoptosis. The manner by which resveratrol inhibits cell growth probably involves a combination of induction of apoptosis and disruption of cell cycle control. The effects of RES on cell viability and proliferation appear to be concentration and cell-type dependent. Whereas high (15–100 μM) concentrations of RES were required to inhibit proliferation and to induce death in cell lines, low concentrations (<10 μM) were cytostatic without a significant effect on cell growth [50–53]. The effects of RES also differ depending on the ER status of breast cancer cell lines. Pozo-Guisado *et al.* [52] showed that although RES decreased cell viability and proliferation in both breast cancer cell lines MCF-7 (ER +) and MDA-MB-231 (ER–), apoptotic cell death was only induced in MCF-7. The characteristics of the target cell may determine not only the effects of varying concentrations of RES on cell proliferation but also the activation of mechanisms leading to apoptotic or nonapoptotic death.

2.4.1 Effects on cell cycle

RES has been demonstrated to directly inhibit the proliferation and viability of human breast cancer cells *in vitro*, as manifested by significant reduction of cells in G_1 with cell accumulation in the S-phase, which correlated with inhibition of ribonucleotide reductase activity by RES [52, 54–57]. The cell cycle is normally controlled by a number of

proteins including p53, $p21^{waf1}$, the cyclin-dependent kinases (cdks) and their activators, the cyclins. RES (10–150 μM) differentially affects the protein expression and kinase activity of G_1/S (cyclin D1/CDK4 and cyclin E) and G_2/M (cyclin B1/cdc2) regulators between MCF-7 and MDA-MB-231, particulary at low concentration. In MDA-MB-231, RES induced a concentration-dependent inhibition in the expression and kinase activity of G1/S and G2/M regulators. In contrast, MCF-7 cells exhibited a transient increase in the expression and kinase activity of these proteins that peaked at 50 μM RES. At concentrations of RES higher than 50 μM, the expression and activity of these proteins returned to close to control levels, with a pattern of decay similar to that observed in MDA-MB-231 [52]. On the other hand, a recent report described a marked inhibition of D-type cyclins (D1 and D2 cyclins) and Cdk4 levels in MCF-7 treated by 50 μM RES [56]. RES (12.5–50 μM) was also shown to increase both protein and mRNA levels of *$p21^{waf1}$* and *p53* in MCF-7 [52, 56, 58]. Considering that $p21^{waf1}$ has been shown involved in cell cycle arrest by RES [59], Pozo-Guisado *et al.* [52] suggested that in MCF-7 cells, the activation of the G_1/S transition by increased kinase activity and $p21^{waf1}$ induction could result in cell accumulation in S phase.

2.4.2 Effects on PI3K signaling pathway

In a recent report, RES was described to regulate ERα-associated PI3K activity in MCF-7 cells [53]. From 10–150 μM, RES induced a biphasic pattern of PI3K activity that increased at low concentrations around 10 μM and decreased at concentrations greater than 50 μM. The activation of downstream effectors PKB/AKT closely followed the profile of PI3K activity [53]. Active PKB/AKT phosphorylates an array of proteins controlling cell survival, growth, proliferation, and apoptosis [60, 61]. Activated PI3K supports cell survival pathways by promoting entry into the S-phase and cyclin D1 expression in MCF-7 cells [62]. The activation of the PI3K pathway by low concentrations of RES in MCF-7 cells resulted in increased GSK3 phosphorylation and elevated levels of cyclin D1 protein [53]. Interestingly, this PI3K-mediated increase in cyclin D1 levels could help explain the increase in cyclin D1/CDK4 kinase activity and cell accumulation at the S-phase previously observed in MCF-7 cells treated with concentrations of RES below 50 μM [52]. On the contrary, high concentrations of RES consistently inhibited PI3K and PKB/AKT activities and decreased GSK3 phosphorylation in MCF-7 cells [53]. These coordinated effects could account for the lower cyclin D1/CDK4 kinase activity, cell cycle blockade, and increase in apoptosis reported in this cell line at concentrations of RES between 50 and 150 μM [52]. The authors concluded that the PI3K pathway in MCF-7 cells may contribute to the antiproliferative and pro-apoptotic activities of RES [53].

2.4.3 Effects on apoptosis

The RES-induced decrease in cell proliferation also resulted in increased rates of cell death. RES, through various regulatory mechanisms, has been shown to induce apoptosis in breast cancer cell lines. Apoptosis induction seems to be cell type-specific, as apoptotic cell death was induced in MCF-7 cells but not in MDA-MB-231 cells [52]. The tumor suppressor p53 inhibits cell growth through activation of cell cycle arrest and apoptosis. RES-induced activation of p53 and apoptosis depends on the activities of ERKs and p38 kinase and their phosphorylation of p53 at serine 15 [63, 64]. A recent report observed that RES (10 μM) induced MAPK activation (Erk1/2), serine phosphorylation, and acetylation of p53 and p53-dependent apoptosis in MCF-7 cells [65]. RES-induced serine phosphorylation of p53 in cancer cells is essential to RES-induced apoptosis [63, 66, 67] and acetylation of p53 allows p53-dependent gene expression [68].

P53-mediated apoptosis mainly occurs *via* transcriptionally dependent and independent mechanisms that activate the mitochondrial pathway [69]. Transcriptional activation of mitochondrial proteins, such as Bax causes apoptosis. p53 downregulates Bcl-2, which acts as an anti-apoptotic agent [70]. Interactions among the Bcl-2 family proteins (Bax, Bak, Bcl-2, Bcl-X, *etc.*) stimulate the release of cytochrome *c* to induce apoptosis [71, 72]. Increased expression of Bax can induce apoptosis by suppressing the activity of Bcl-2 [73, 74]. This means that the ratio of Bcl-2 to Bax is important for the apoptosis induced by chemoprevention agent [75]. RES (12.5–74 μM) increases the expression of apoptotic Bax and Bak and downregulates anti-apoptotic Bcl-2 and Bcl-x_L in MCF-7. The increased ratio of Bax to Bcl-2 might contribute to apoptosis induction in RES-treated MCF-7 cells [51, 56].

Caspases are crucial components of the apoptosis pathway [76]. The crucial step in activation of the cell death program is activation of caspase 3 by caspase 9. RES (52–74 μM) activates caspase 3 in the MKL-F breast cancer cell line, which is a derivative of MCF-7 [51]. It also induces caspase 9 activity in MCF-7 [56], which lacks procaspase-3 due to the deletion of the caspase 3 gene [77, 78]. The p53 protein can also enlist other effector genes. It can activate the death receptor pathway *via* receptor target genes, such as CD95. In T47D breast carcinoma cells, RES (32 μM) seems to trigger CD95/CD95L-signaling-dependent apoptosis resulting from an increase in cell surface expression of CD95L [79]. In conclusion, in breast cancer cell lines, the antiproliferative activity of RES seems to result from the induction of apoptotic cell death and disruption of cell cycle control (Fig. 4).

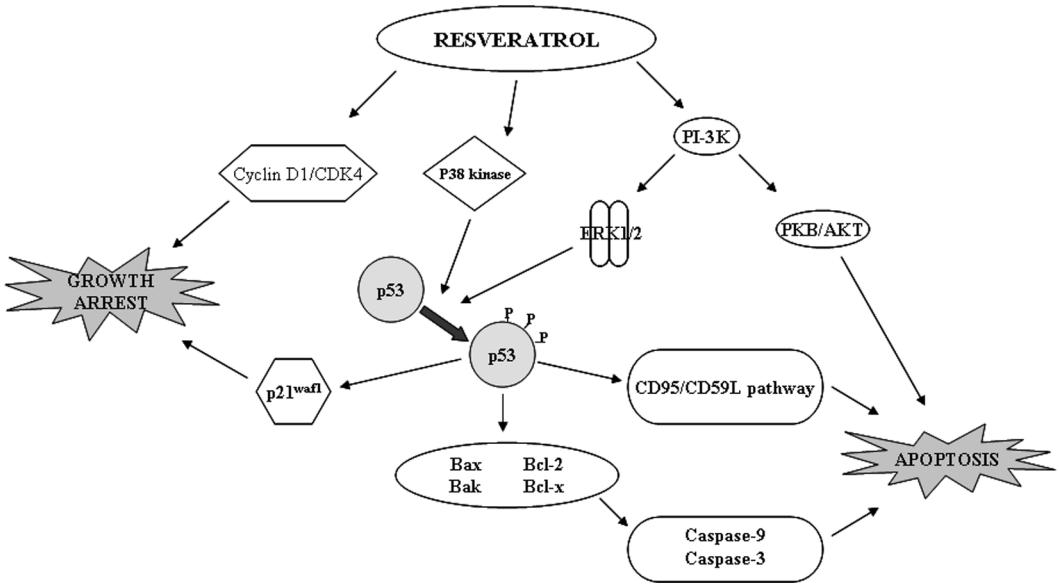

Figure 4. Antiproliferation effects: resveratrol induces different pathways leading to cell growth arrest and apoptosis.

2.5 Other chemoprevention effects of RES in breast cancer

In our previous work, we investigated the effects of RES on BRCA1 and BRCA2 protein and mRNA expression in human breast cancer cell lines (MCF7 and MDA-MB 231). The expression of *BRCA1* and *BRCA2* mRNAs increased although no change in the expression of the proteins was found [55]. Germ-line mutations in the breast susceptibility genes *BRCA1* and *BRCA2* confer a high risk for developing breast and ovarian cancer [80, 81]. BRCA1 and BRCA2 regulate multiple nuclear processes including DNA repair, cell cycle checkpoints and transcription [82]. We found that RES (30 – 50 μM) regulates mRNA expression of genes (*p53*, *p21*$^{WAF1/CIP1}$, *ERα, ERβ*, *RAD51*, and *CBP/P300*) implicated in BRCA1 and BRCA2 functions in ER-positive (MCF-7) and ER-negative (MDA-MB-231 and MCF-10a) breast cell lines in both ER-dependent and independent manners [58]. These data indicate that RES can increase expression of genes involved in the aggressiveness of human breast tumor cell lines. RES (6 – 100 μM) was also shown to inhibit angiogenesis *in vitro* [83]. The growth of solid tumors and metastases depend on tumor angiogenesis [84]. The inhibition of angiogenesis *in vivo* can attenuate tumor growth and metastasis [85].

In a recent report, RES (30 μM) was found to be a potent sensitizer for anticancer drug-induced apoptosis in a variety of human tumor cell lines by inducing cell cycle arrest and survivin depletion in a p53-independent manner [86]. Survivin is an inhibitor of apoptosis proteins (IAPs), which are highly expressed in many tumors and have been associated with refractory disease and poor prognosis [87, 88]. Inhibition of effector caspases by IAPs occurs at the core of the apoptotic machinery. Thus, therapeutic modulation of IAPs could target a key control point in cancer resistance [89]. Another study suggested that RES (0.4 – 4 μM) may affect breast cancer cell sensitivity to vitamin D_3 analogs through regulation of the vitamin D_3 receptor (VDR) promoter. Both T47D and MCF-7 cells pretreated with RES exhibited increased VDR-mediated transactivation of vitamin D_3-responsive promoter [90]. The steroid hormone 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) binds the VDR and this complex regulates the transcription of genes involved in cell cycle, apoptosis, and differentiation. In breast cancer, $1,25(OH)_2D_3$ causes growth arrest and apoptosis *in vitro* and *in vivo* [91].

2.6 *In vivo* effects of RES

Currently, few studies were available on the potential *in vivo* efficacy of RES in animal models of breast tumorigenesis. Previously, estrogenicity of RES was investigated in rats by determining whether RES is an estrogen agonist on selected reproductive and nonreproductive estrogen-target tissue. RSV treatment (1 – 100 μg/day) was described to have minimal *in vivo* effects on estrogen target tissues in rats, including no effect on uterine growth and differentiation, body weight, serum cholesterol, or radial bone growth. In contrast, RES antagonized the effects of estrogen to lower serum cholesterol. The authors suggested that RES has little or no estrogen agonism but may be an estrogen antagonist [92]. Likewise, RES (0.03 – 575 mg/kg/day) was

concluded to be inactive in immature female Alpk:ApfSD and Sprague-Dawley rat uterotrophic assays [93, 94]. In view of these observation, further investigations are necessary to evaluate the estrogenic potential of RES.

Others studies examined the effects of RES in mammary tumor models. Banerjee *et al.* [95] found that RES (10–100 mg/kg/day) inhibited the early stages of *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis when orally administrated to female Sprague-Dawley rats. It caused a significant suppression of tumor multiplicity and tumor incidence was reduced by ~50%. Likewise, Banerjee *et al.* [46] reported that RES (100 μg/rat) fed to Sprague-Dawley female rats inhibited tumor formation in 7,12-dimethylbenz[a]anthracene (DMBA)-initiated mammary carcinogenesis. Tumor incidence and multiplicity were reduced by ~50%. RES-induced inhibition was shown in correlation with downregulation of NF-κB, COX-2, and matrix metalloprotease-9 expression. On the other hand, RES (1–5 mg/kg/day) appeared to have no effect on the growth of 4T1 mammary tumor in BALB/c female mice [96]. In contrast, short RES treatment (100 mg/kg/day) of prebubertal Sprague-Dawley female rats affected endocrine function and accelerated development of MNU-induced mammary carcinoma with an increase of tumor incidence and multiplicity [97].

Potential chemoprevention of RES also depends on its safety and toxicity. In the studies previously described, used RES concentrations appeared not harmful for the rats. Furthermore, Juan *et al.* [98] described the effects of the repeated oral administration of RES (20 mg/kg/day) to Sprague-Dawley rats. RES did not affect mortality, hematologic tests, organ weight, and no pathologic signs were observed in the vital organs during the experimental period. Only very high doses of RES (1000–3000 mg/kg/day) induced renal toxicity in rats by increasing kidney weight and renal lesions, such as an increased incidence and severity of nephropathy [99]. Thus, moderate absorption of RES has no toxicity and seems to exhibit a chemoprevention effect *in vivo* by inhibiting the early stages of breast tumorogenesis.

3 Conclusions

Chemoprevention of carcinogenesis by nontoxic chemical substances is regarded as a promising alternative strategy to therapy for control of human cancer. In recent years, many naturally occuring substances have been shown to protect against experimental carcinogenesis. In this regard, the present review summarizes the evidence which suggest that RES is a potent chemopreventive agent in breast cancer. RES provides cancer chemopreventive effects in different systems based on its inhibition of diverse cellular events associated with tumor initiation, promotion, and progression. Although RES has shown remarkable promise as a potent chemopreventive agent in breast cancer, there is a long way to go for it to be developed as an agent for chemoprevention/treatment of cancer. Continued efforts are needed, especially well-designed preclinical studies in animal models.

We thank Nancy Uhrhammer for correcting the English. Ludovic Le Corre was a recipient of grant from the M.E.N.R.T. (Ministère de l'Education Nationale de la Recherche et de la Technologie).

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