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Resveratrol as a Chemopreventive Agent: A Promising Molecule for Fighting Cancer

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Abstract: Resveratrol (3,4',5 tri-hydroxystilbene) is a phytoalexin produced in huge amount in grapevine skin in response to infection by *Bothrytis cinerea*. This production of resveratrol blocks the proliferation of the pathogen, thereby acting as a natural antibiotic.

Numerous studies have reported interesting properties of *trans*-resveratrol as a preventive agent against important pathologies i.e. vascular diseases, cancers, viral infection or neurodegenerative processes. Moreover, several epidemiological studies have revealed that resveratrol is probably one of the main microcomponents of wine responsible for its health benefits such as prevention of vaso-coronary diseases and cancer.

Resveratrol acts on the process of carcinogenesis by affecting the three phases: tumor initiation, promotion and progression phases and suppresses the final steps of carcinogenesis, i.e. angiogenesis and metastasis. It is also able to activate apoptosis, to arrest the cell cycle or to inhibit kinase pathways. Interestingly, resveratrol does not present any cytotoxicity in animal models. Moreover, concentrations of resveratrol in blood seem to be sufficient for anti-invasive activity. The enterohepatic recirculation may contribute to a delayed elimination of the drug from the body and bring about a prolonged effect. By its binding to plasmatic proteins, resveratrol also exhibits a prolonged effect. Interestingly, low doses of resveratrol can sensitize to low doses of cytotoxic drugs and so provide an innovative strategy to enhance the efficacy of anticancer therapy in various human cancers. By these properties, resveratrol appears to be a good candidate in chemopreventive or chemotherapeutic strategies and is believed to be a novel weapon for new therapeutic strategies.

Key Words: Resveratrol, cancer, chemoprevention, sensitization, bioavailability.

A) INTRODUCTION

Resveratrol or 3, 5, 4' tri-hydroxystilbene (Fig. 1) is a secondary metabolite produced in limited plant species. The root of the word "resveratrol" is a combination of the latin prefix *Res*, meaning "which comes from", *veratr*, from the plant "Veratrum", and the suffix *ol*, indicating that it contains "alcohol" chemical groups. *Veratrum grandiflorum* has been reported to synthesize resveratrol and analogues. It is not uninteresting to note that root powder of *Veratrum album* has long been used for at medium altitude in Northern Europe, Asia and Japan to treat rheumatism and nervous diseases. However, *Veratrum album* contains potent toxic alkaloids: the protoveratrin A & B. The resveratrol precursor is phenylalanine and the key cell enzyme is stilbene synthase which orientates the synthesis pathway toward resveratrol, instead of toward flavonoids through chalcone synthase [1]. Therefore, resveratrol can be classified either as a stilbene or as a polyphenol. Several plant species are known to produce resveratrol (especially the *trans* isomers such as aglycone or in a glycosylated form), in significant to high amounts. Some of them are used as food, i.e. vine plant, peanuts, berries. In the vine plant, *Vitis vinifera*, resveratrol

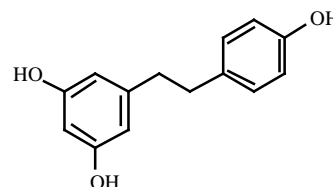


Fig. (1). Chemical structure of resveratrol (3,5,4'-trihydroxystilbene in classical nomenclature).

is a phytoalexin, i.e. produced in huge amounts in grape vine skin in response to infection by *Bothrytis cinerea*, leading to a blockage of its proliferation. Obviously, resveratrol appears to be a real natural antibiotic. Other resveratrol producing plants are not used as food, i.e. *Polygonum cuspidatum* or *Yucca schidigera*. In ancient Chinese natural medicine, extracts of *Polygonum cuspidatum* were used for their vaso-relaxing activity, while root extracts of *Yucca schidigera* were known for their anti-mutagen activity [2].

Like many other plant polyphenols, resveratrol is considered to be preventive food microcomponent as are the flavonoids and epicatechins of green tea or cocoa [3]. Indeed, numerous studies have reported interesting properties of *trans*-resveratrol as a preventive agent of several important pathologies: vascular diseases, cancers, viral infection, neurodegenerative processes such as Alzheimer's [4] (for reviews, see [2,5,6]). Resveratrol is also a potent antioxidant as

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shown by the LDL protection against oxidation. In addition, as recently reported, resveratrol may also increase lifespan [7]. Moreover, several epidemiological studies (in particular [8]) revealed that resveratrol may be one of the main wine microcomponents responsible for the health benefits (i.e. against vaso-coronary diseases and cancer mortality) in the case of moderate wine consumption. Moreover, due to its oestrogeno-mimetic properties, resveratrol may protect women against osteoporosis [9]. The resveratrol antiproliferative effect has been shown *in vitro* in several cell lines derived from tumors and the resveratrol anticarcinogenic effects are demonstrated in several animal models. In this review, we detail the mechanism of the effects of resveratrol in different steps of carcinogenesis chemoprevention. Indeed, resveratrol is able to prevent tumor initiation by scavenging for free radicals damaging DNA and by the activation of detoxifying enzymes. Resveratrol also inhibits tumor promotion by the modulation of polyamine metabolism and tumor progression, by the modulation of the cell cycle and by the induction of apoptosis. We and others have observed that resveratrol can induce apoptosis and an arrest of the cell cycle. In this review, we detail the mechanism of cell cycle arrest and the apoptosis mechanism in adenocarcinoma colon. Resveratrol can also sensitize various cancer cells to several apoptotic drugs, which would be interesting property for clinical trials.

B) RESVERATROL AND CARCINOGENESIS

Dietary polyphenols is of great interest due to their anti-oxidative and anticarcinogenic activities. Indeed, polyphenols can have a chemoprotective effect which is the property of pharmacological or natural agents that promote the arrest or regression of a cancer process. Polyphenols such as resveratrol may inhibit carcinogenesis by affecting the molecu-

lar events in the initiation, promotion and progression stages (Fig. 2).

1. Resveratrol and Tumor Initiation

Resveratrol could act on carcinogenesis by inhibiting the initiation phase which consists of the DNA alteration (mutation) of a normal cell, which is an irreversible and fast change. The initiated cell is capable to autonomous growth. The initiating event can consist of a single exposure to a carcinogenic agent or in some cases, it may be an inherited genetic defect. The anti-initiation activity of resveratrol is linked to the suppression of the metabolic activation of carcinogens and/or the detoxifying increases *via* a modulation of the drug-metabolizing enzymes involved either in phase I reactions transforming a lipophilic compound into an electrophilic active carcinogen, or in phase II conjugation enzyme systems converting the primary metabolite into a final hydrosoluble metabolite (Fig. 2).

a) Resveratrol Chemoprevention by Inhibition of Phase I Enzymes

Firstly, resveratrol could exert a chemopreventive action against polycyclic aromatic hydrocarbon-induced carcinogenesis. Indeed, it has been established that compounds such as polycyclic aromatic hydrocarbons (PHA) or nitrosamines are procarcinogens and have to be metabolically activated into electrophilic active carcinogens. Interaction of these electrophilic compounds with genomic DNA forms DNA adducts and contributes to induce tumor initiation through oncogen activation (Fig. 2). So, resveratrol is able to reduce the number of DNA adducts induced by various chemical agents. An example of this is the reduction of the number of benzo[a]pyrene (B[a]P)-induced DNA adducts in human bronchial epithelial cells [10-13]. This effect is correlated

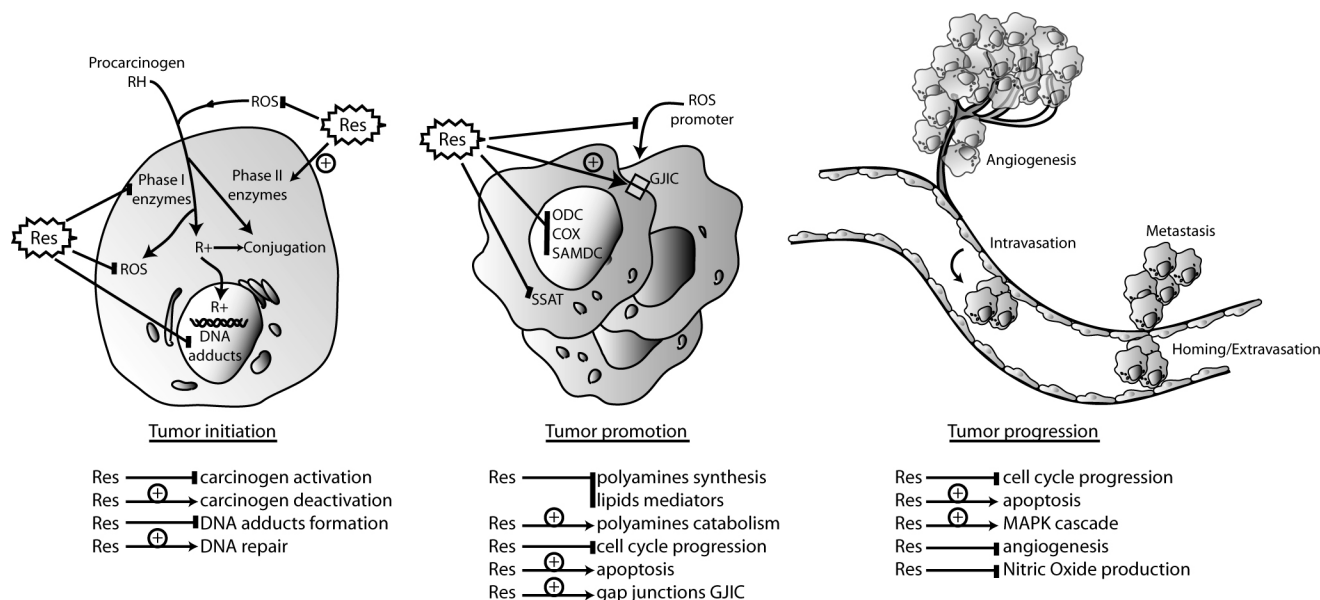


Fig. (2). Resveratrol effects on multistage carcinogenesis. Resveratrol is able to prevent initiation phase by inhibition of carcinogen activation (R+) induction of carcinogen deactivation and subsequently blocking interaction between DNA and carcinogen (R+). Resveratrol can block the action of tumor promoter, and can act on tumor progression by inhibition of angiogenesis and metastatic process.

with a decrease of B[a]P-derived metabolic products and with the inhibition of cytochrome P450 1A1 (P450 1A1) and P450 1B1 gene expression [10]. It seems that resveratrol can prevent metabolic activation of procarcinogens by a competitive inhibition of the aryl hydrocarbon receptor (AhR). Resveratrol antagonizes the transactivation of genes regulated by AhR ligand, such as PHA (B[a]P; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); 7,12-dimethylbenz[a]anthracene (DMBA)) in various cell systems [14-17]. But the ability of resveratrol to bind to AhR and act as a competitive antagonist of this receptor is controversial. Indeed, some authors have shown that resveratrol inhibits P450 1A1 *via* an AhR-independent post-transcriptional pathway [18]. The results would differ according to the cell systems or the methodology used. AhR is involved in various processes such as cell proliferation, differentiation and P450 1A induction after xenobiotics exposure. This enzyme, P450 1A1, which is well known as an aryl hydrocarbon hydroxylase, is often considered to be one of the most important enzymes involved in tumor initiation. Thus, one possible mechanism through which phenolic compounds might exert anticarcinogenic effects is an interaction between them and the P450 system, either by the inhibition or the activation of certain forms of this enzyme, leading to a reduced production of the ultimate carcinogen [19]. Frotschl (1998) was the first to show the effect of resveratrol on P450: resveratrol induces P450 1A1 mRNA in human Hela cell cultures [20]. However, it appears that resveratrol decreases the ARNm basal level of P450 1A1 and P450 1A1 promoter activity [21], as well as P450 1A1 and P450 1A2 enzyme activities [14,22] in various cell types such as hepatoblastoma, breast carcinoma or the human bronchial epithelial cell line BEP2D [23,24]. Resveratrol could act on P450 1A2 *via* antagonizing AhR properties because P450 1A2 is regulated partly by the AhR system [25].

Resveratrol is able to inhibit alkoxyresorufin O-dealkylase (AROD) activities for various isoforms of P450. Resveratrol is also able to inhibit benzylresorufin O-demethylation (MROD), ethoxyresorufin O-deethylation (EROD) activity in human liver microsomes and methoxyresorufin O-demethylation (MROD) [26,27]. This inhibition of EROD and MROD activities concerns in particular P450 1A1 (inhibition competitive / non-competitive). However, the activities of human NADPH-P450 reductase are not significantly changed by resveratrol [26]. Other P450 enzymes involved in the metabolic activation of many pollutants and in the development of various forms of cancers are affected by the inhibitory effect of resveratrol, as is the case for P450 1B1 [10,23,28], P450 3A4 [29-31], P450 3A5 [31].

These resveratrol actions are also shown *in vivo*. For example, in mice, administration of resveratrol abrogates DNA adduct induction by B[a]P and decreases the expression of P450 1A1 [17,32-34].

b) Resveratrol Chemoprevention by ROS Scavenging

Reactive oxygen species (ROS) arise whenever the cell is involved in oxygen utilization, and this production may be exacerbated by xenobiotic drugs. These ROS actively participate in the metabolic activation of procarcinogens and the events associated with the process of carcinogenesis such as oncogene mutation, by modifying the structure of DNA bases (Fig. 2). The inhibition of P450 by resveratrol can re-

duce the reactive activation of molecular oxygen. Resveratrol is able to prevent the increase in ROS following exposure to oxidative agents such as tobacco-smoke condensate (TAR), H₂O₂, phorbol esters, ultraviolet radiation [35-37], and to decrease and scavenge ROS [38,39]. It appears that resveratrol is an effective scavenger of hydroxyl, superoxide, and metal-induced radicals. Resveratrol exhibits a protective effect against lipid peroxidation in cell membranes and DNA damage caused by ROS [38]. The antioxidative effect of resveratrol is also shown in rats where a pretreatment by the polyphenol prevents oxidative damage in renal DNA of rats treated with the kidney carcinogen KBrO₃ [40]. It is also the case against lipid peroxidation where a single local application of resveratrol to SKH-1 hairless mice inhibits the increased levels of lipid peroxidation induced by UVB [41]. These antioxidant actions of resveratrol contribute to prevent oxidative DNA damage which plays a pivotal role in the carcinogenic activity of many genotoxic agents.

c) Resveratrol Chemoprevention by Induction of Phase II Enzymes

Phase II enzyme induction generally protects tissues and cells from endogen and/or exogen intermediate carcinogens. Phase II conjugation reactions lead to the formation of a covalent linkage between a functional group on the parent compound and glucuronic acid, sulfate, glutathione, amino acids, or acetate (Fig. 2). Resveratrol can inhibit the activities of *O*-acetyltransferase and sulfotransferase in breast cancer cell lines, contributing to reduced DNA adduct formation [42]. This phenomenon is also observed in normal human mammary epithelial cells, where resveratrol reduces estrogen sulfotransferase activity in a competitive manner [43]. Conversely, resveratrol contributes to metabolic inactivation by inducing UDP glucuronosyltransferase (by approximately 100-150%) and to a lesser extent NAD(P)H:quinone oxidoreductase (NQO1) [13,44]. Resveratrol also increases glutathione (GSH) levels and the activity of glutathione-S-transferase (GST) as well as the activity of glutathione peroxidase (GPX) and glutathione reductase (GR) in various cells (e.g. Chinese hamster ovarian cells, human lymphocytes) and subsequently reduces DNA damage [45-47]. It is interesting that non-tumoral cells such as normal human peripheral blood mononuclear cells (PBMNCs) acquire an antioxidant capacity when treated with resveratrol against an apoptogenic oxidant such as 2-deoxy-D-ribose [48]. These actions on the enzymatic systems were also shown *in vivo*: in a mouse-skin model treated with (12-*O*-tetradecanoylphorbol-13-acetate) TPA, a pretreatment with resveratrol restores glutathione levels as well as myeloperoxidase, oxidized glutathione reductase and superoxide dismutase activities to control levels [49]. Resveratrol could activate the phase II detoxifying enzyme gene expression *via* a modulation of the mitogen-activated protein kinases (MAPK) pathway. Indeed, Kong *et al.* have proposed a model where an antioxidant such as butylated hydroxyanisole (BHA) and isothiocyanate sulforafane (SUL) may modulate the mitogen-activated protein kinases (MAPK) pathway leading to transcriptional activation of the nuclear factor erythroid 2p45 related factor, Nrf2 (a basic leucine zipper transcription factor) and of the antioxidant electrophile response element (ARE), with subsequent induction of phase II detoxifying enzymes such as GST, NQO-1 [50]. A recent study shows that resveratrol is

able to up-regulate NQO-1 gene expression in human ovarian cancer PA-1 cells [51]. A possible hypothesis is that resveratrol, by its activation of kinase pathways (see below), could activate GST and NQO-1 gene expression and the subsequent detoxifying activities. Furthermore, a study using a resveratrol affinity column shows that the dihydronicotinamide riboside quinone reductase 2 (NQO2) binds resveratrol and could constitute a potential target in cancer cells [52].

d) Resveratrol Chemoprevention by the Stimulation of DNA Repair

Many drugs and ultraviolet irradiation cause DNA damage and failure to repair this damage results in carcinogenesis. This DNA alteration presents an obstacle to DNA polymerase. In order to protect against the effects of mutational rates, several genes such as p53 have to survey the genome damage and / or to repair these damages. Resveratrol is able to stimulate DNA repair by increasing the activity of p53 [53] in various cell lines. It was also reported that resveratrol as well as other natural products (curcumin, ellagic acid, ...) help in the recovery of DNA damage by accelerating DNA repair efficiency in the damaged cells [54]. The SOS suppression and antimutagenicity of resveratrol were shown against Trp-P-1 in *Salmonella thyphimurium* [55]. Furthermore, resveratrol is able to activate egr-1 transcription and it seems that an enhancement of Egr-1 protein levels may be needed to regulate genes involved in DNA repair and cell survival or apoptosis (see below resveratrol and cell death)

2. Resveratrol and Promotion

The initiated cell may remain dormant for months or years and unless a promoting event occurs, it may never develop into a clinical cancer case. The promotion phase is the second major step in the carcinogenesis process in which specific agents (*referred to as promoters*) trigger the further development of the initiated cells. Promoters often, but not always, interact with the cellular DNA and influence the further expression of the mutated DNA so that the initiated cell proliferates and progresses further through the carcinogenesis process (Fig. 2).

Resveratrol potentially antagonizes tumor promotion in the DMBA/TPA mouse skin carcinogenesis model [17,49]. The administration of the polyphenol to female Sprague Dawley rats was able to reduce mammary tumorigenesis induced by different promoters such as N-methyl-N-nitrosourea [56], DMBA [57], and to reduce N-nitrosomethylbenzylamine (NMBA)-induced rat esophageal tumorigenesis [58]. The primary target mediating the tumor-promoting activity of the phorbol ester such as TPA is the protein kinase C (PKC) family.

a) Resveratrol Chemoprevention by Inhibition of Kinase Cascade

Resveratrol is able to act on the MAPK cascade *via* several kinases (Fig. 5). Indeed, resveratrol is able to act on the preceding stages by inhibiting the phosphorylation and the activity of PKC [59-62]. Resveratrol inhibits the PKC-catalyzed phosphorylation of arginin-rich protein substrate in a non competitive manner [63]. The potency of resveratrol depends on the nature of the substrate and cofactors [63]. Like diacylglycerol, resveratrol interacts with the C1 do-

main and induces the association of PKC with membrane vesicles. Resveratrol is able to block many PKC isoenzymes, such as cPKC, nPKC, PKC [63,64] and inhibit the PKC activation induced by phorbol-esters such as phorbol myristate acetate (PMA) [65]. However, resveratrol is not able to inhibit PKC isoenzyme autophosphorylation, whereas it can inhibit the autophosphorylation of protein kinase-D (PKD) [66-68]. However, very high concentrations of resveratrol are required to achieve inhibition of PKD autophosphorylation and activity [68]. This PKD is a key player of the nuclear factor kappa B (NF B) pathway in which it relays a signal from ROS to the activation of canonical IKK/NF B signalosome. When the PKD/NF B pathway is blocked, cells are less protected from ROS-induced apoptosis. Specifically, resveratrol blocks PKD activation loop phosphorylation and activity, and this is due to a specific inhibition of the PKC [69]. Conversely, resveratrol does not affect Abl kinase activity [69]. So, this action contributes to the resveratrol-induced NF B pathway blockage.

Resveratrol can also exert a dual effect on the MAPK cascade activation. Indeed, the polyphenol can inhibit the MAPK pathway activation mediated by various promoters [70,71]. Conversely, resveratrol can induce the activation of the same pathways to activate the cell death pathway, which could be interesting to fight the progression stage (see below resveratrol and progression) [72-76]. For example, *in vitro*, resveratrol inhibits PMA-mediated activation of c-Jun N-terminal kinase (JNK) (Fig. 5) [65]. *In vivo*, pretreatment of the dorsal skin of female ICR mice with resveratrol decreases the phosphorylation of extracellular signal-regulated protein kinase (ERK) as well as the catalytic activity of ERK and p38 MAPK stimulated by various stimuli [71,77,78]. In addition, resveratrol prevents TPA-induced DNA binding of activator protein-1 (AP-1) [77]. The inhibition of the tyrosine phosphorylation on kinase and their translocation into the nucleus from the cytoplasm reduces the expression of various genes implicated in the proliferation, differentiation and angiogenesis (Fig. 5) [79]. So, by its blocking action of the stimuli-mediated MAPK pathway activation, resveratrol could possess an antitumor-promoting property.

b) Resveratrol Chemoprevention by Inhibition of Polyamine Synthesis

The inactivation of PKC by resveratrol could contribute to inhibit ornithine decarboxylase (ODC) gene expression, which encodes the enzyme required for the first stage in polyamine synthesis. In normal cells, ODC and several polyamine metabolic proteins are essential, but in many cancers arising from epithelial tissues, such as the skin and colon, ODC and polyamine levels are increased. Polyamines affect numerous processes in carcinogenesis such as promotion, progression and invasion. Suppression of polyamine levels is associated with decreased cell growth, and increased apoptosis. It appears that resveratrol can inhibit polyamine synthesis and increase polyamine catabolism. Indeed, several reports have shown that resveratrol can significantly decrease ODC mRNA in colon cancer lines and protein levels as well as activity [80,81]. This reduction of polyamine synthesis is reinforced by the inhibitory action of resveratrol on S-adenosylmethionine decarboxylase (SAMDC) which synthesizes higher polyamines [81]. On the other hand, resveratrol

boost the activity of spermidine/spermine N(1)-acetyltransferase (SSAT), which is a rate-limiting enzyme in polyamine catabolism that degrades polyamines in cooperation with polyamine oxidase [81]. These data are also shown *in vivo* where the local application of resveratrol on mice significantly reduces UVB-induced increased ODC activity and protein expression [41,82]. Transcriptional activity of human *odc* gene is directly mediated by the Myc/Max transcriptional complex [83]. It appears that there is a correlation between the inhibition of *odc* gene expression by resveratrol and a reduction of the transcription factor c-Myc [81].

c) Resveratrol Chemoprevention by Inhibition of Lipid Mediators

The lipid mediators such as prostaglandins (PGs) have been shown to be involved in promoting cell proliferation, suppressing immune surveillance, and stimulating tumorigenesis [84]. The synthesis of these products from arachidonic acid can occur *via* to several pathways such as the prostaglandin H synthase (PHS) pathway, the cyclooxygenase (COX), and the lipoxygenase pathways.

Prostaglandin H synthase (PHS) is the primary enzyme responsible for the biosynthesis of prostaglandins and thromboxanes. Resveratrol is a competitive inhibitor of cyclooxygenase and peroxidase activity of PHS in human erythroleukemia cells [85,86]. As far as PHS is concerned, both cyclooxygenase and peroxidase activities depend on ferriprotoporphyrin IX [87,88]. Again, the prolonged lag phase of the cyclooxygenase reaction was indicative of a reduction of Fe(III) to Fe(II) [88,89]. The cyclooxygenase inhibition by resveratrol prevents the release of cyclooxygenase products such as prostaglandins and thromboxanes [86,90-95]. The mechanism of PHS inhibition by resveratrol has yet to be firmly established because there has been published a contradictory study showing that resveratrol is a non-competitive inhibitor of the peroxidase activity of the first isoenzyme of PHS, PHS-1, which is constitutively expressed, but that resveratrol does not inhibit the cyclooxygenase activity of PHS-2 which is induced by mitogen and various stimuli [96].

Resveratrol can inhibit the hydroperoxidase function of COX which can lead to anti-initiation activity and can also inhibit the production of arachidonic acid metabolites catalyzed by COX-1 or COX-2, contributing to its antipromotion activity [97]. COX-1 and COX-2 are respectively constitutive and inducible enzymes that catalyze the production of pro-inflammatory prostaglandins (PGs) from arachidonic acid. Various reports show that resveratrol inhibits COX-1 and COX-2 activity [44,98,99]. In fact, resveratrol discriminates between both COX isoforms. The polyphenol is a potent inhibitor of both catalytic activities (cyclooxygenase and hydroperoxidase) of COX-1. In fact, resveratrol non-competitively inhibits the cyclooxygenase activity of COX-1 [100]. A recent study has shown that resveratrol-inactivated COX-1 was devoid of both cyclooxygenase and peroxidase activities, this inactivation being accompanied by a concomitant oxidation of resveratrol [101]. Moreover, docking studies on both COX-1 and COX-2 protein structures also revealed that hydroxylated but not methoxylated resveratrol analogues are able to bind to the previously identified binding sites of the enzymes [102]. The peroxydase activity of

COX-2 is the isoform target for nonsteroidal anti-inflammatory drugs. Resveratrol inhibits PKC, ERK1 and c-jun induced COX-2 promoter activity [103-106], and resveratrol also directly inhibits the activity of COX-2 [103]. Various reports have demonstrated that eukaryotic transcription factor such as AP-1 or NF- κ B are involved in the regulation of COX-2. Resveratrol is able to reduce the DNA binding activity and transcriptional activities of nuclear factors [81,107,108] and subsequently decreases the transcriptional activity of COX-2 expression [106,109]. These results are also shown *in vivo* where administration of resveratrol is able to suppress NMBA-induced rat oesophageal tumorigenesis by targeting COXs and PGs production [58]. Indeed, high levels of expression of COX-1 in tumor tissues, increased COX-2 expression and the increased levels of PGE₂ synthesis were decreased by the administration of resveratrol [58]. The local application of resveratrol on mice significantly inhibits UVB-induced increased protein levels and the activity of epidermal COX-2 [41,82]. In Apc(Min+) mouse, several studies have produced contradictory results concerning a decrease in PG production as well as in COX activity after administration of resveratrol [110,111].

Resveratrol is able to act on the lipoxygenase family. In the presence of the substrate (linoleic acid), resveratrol inhibits both 5-lipoxygenase and 15-lipoxygenase as a competitive inhibitor [85,112]. Resveratrol prolongs the lag phase of both enzymes, indicating a possible reduction of Fe(III) to Fe(II) at the catalytic site [96]. Pinto *et al.* have shown that resveratrol inhibits the dioxygenase activity of lipoxygenase and is simultaneously oxidized by the peroxidase activity of lipoxygenase. The oxidized form of resveratrol is a lipoxygenase inhibitor as efficient as the reduced form [112,113]. This lipoxygenase inhibition by resveratrol prevents the release of pro-inflammatory substances [93-95] and consequently blocks the synthesis of heparin, mediators of calcium mobilization, vascular permeability and neutrophil activation [90,114]. Moreover, by inhibiting phospholipase A₂, resveratrol decreases the release of arachidonate from cell lipids and thus the synthesis of metabolites by COX-2 and lipoxygenase pathways [92].

So, this inhibition of pro-inflammatory substances contributes to the anti-inflammatory activity of resveratrol which has been shown in various rat models of carrageenan-induced paw edema [115]. Resveratrol inhibits both acute and chronic phases of this inflammatory process, with an activity greater than that of indomethacin or phenylbutazone. This effect is attributed to the impairment of PGs synthesis *via* selective inhibition of COX-1 [17].

d) Resveratrol Chemoprevention by Cell Cycle Arrest

Resveratrol, like many cytotoxic agents, affects cell proliferation by disturbing the normal progress of the cell cycle. In fact, resveratrol is able to block cell progression through the cell cycle, this blockage varying according to the cell type, the polyphenol concentration, and the treatment duration (Table 1). Resveratrol is able to interfere with the molecular machinery of the cell cycle which involves various key regulators (Fig. 3). Indeed, in eukaryotes, regulation of the cell cycle is controlled in part by a family of protein kinase complexes, and each complex is composed minimally of a catalytic subunit, cyclin dependent kinases (cdks), and its

Table I. Resveratrol Effect on Cell Cycle

| | Cell systems | Cell cycle arrest | Resveratrol effects | References |
|------------------|---|-------------------|---|---------------|
| Solid tumors | Human gastric adenocarcinoma cells (KATO-III, RF-1) | G0/G1 phase | - | [64] |
| | Androgen-nonresponsive human prostate cancer cells (DU-145, PC-3, JCA-1) | G1/S transition | Slightly inhibition of pRb | [39,121] |
| | Human epidermoid carcinoma cells (A431) | G1/S transition | p21 \uparrow ; cyclin D1,D2,E \downarrow ; Cdk2, 4,6 \downarrow ; hyperphosphorylated pRb \downarrow ; hypophosphorylated pRb \uparrow ; E2F(1-5) family \downarrow | [120,123] |
| | Neuroblastoma cells | G1 phase | Survivine \downarrow ; p21 \uparrow | [[131] |
| | Androgen-responsive human prostate cancer cells (LNCaP) | S phase | p21 \downarrow ; p27 \downarrow ; cyclin A,E \uparrow ; cdk2 \uparrow ; inhibition of DNA synthesis | [139,149,189] |
| | Human breast cancer cell line (MCF-7) | S phase | cyclin D \downarrow ; cdk4 \downarrow , p53 \uparrow ; p21 \uparrow | [125] |
| | Human endometrial adenocarcinoma cells (Ishikawa) | S phase | cyclin A, E \uparrow ; cdk2 \downarrow | [142] |
| | Neuroblastoma cells (neuro-2a) | S phase | Cyclin E \uparrow ; p21 \downarrow | [140] |
| | Human lung carcinoma A549 cells | S phase | pRB phosphorylated \downarrow ; p21 \uparrow | [148] |
| | Human hepatoblastoma cell line (HepG2) | S/G2 transition | - | [164] |
| | Human colonic adenocarcinoma cell line (Caco-2) | S/G2 transition | cyclin D1 \downarrow ; Cdk4 \downarrow , cyclin A, E \uparrow ; hyperphosphorylated pRb \downarrow ; hypophosphorylated pRb \uparrow | [80] |
| | Colon carcinoma cell line (HCT-116) | S/G2 transition | cyclin D1 \downarrow ; Cdk4 \downarrow , cyclin A, E \uparrow | [129] |
| | Human adenocarcinoma cell line (SW480) | S/G2 transition | Cyclin A, B \uparrow ; cdk1, 2 hyperphosphorylated \uparrow | [141] |
| | Colon carcinoma cell line (HT29) | G2/M phase | Cdk1 phosphorylated \uparrow ; cdk1 kinase activity \downarrow ; cdk7 kinase activity \downarrow | [168] |
| Non solid tumors | Human acute lymphoblastic (HSB-2) leukemia cells | G1 phase | - | [122] |
| | Human chronic myeloid (K562) | S phase | - | [122] |
| | Lymphocytic leukemia cell line CEM-C7H2 (deficient in functional p53 and p16) | S phase | - | [134] |
| | Acute myeloid leukemia (AML) cell lines (OCIM2 OCI/AML3) | S phase | - | [143] |
| | Human histiocytic lymphoma U937 cells | S phase | cyclin A, D3, E \uparrow ; cdk2 \uparrow , p27 \downarrow | [144,145] |
| | Human promyelocytic leukemia cells (HL-60) | S phase | cyclin A, E \uparrow ; cdk1 phosphorylated \uparrow | [146,147] |

essential activating partner, cyclin. Cyclins play a key regulatory role in this process by activating their partner cdks and targeting them to the respective protein substrates [116]. Complexes-formed in this way are activated at specific intervals during the cell cycle and their inhibition blocks the cell cycle at the corresponding control point.

i) Resveratrol and Arrest in G1 Phase

During mitogenic stimulation, the cell cycle progression machinery is initiated by induction of cyclins D, followed by induction of cyclin E. These events are necessary for activation of cdks, cdk4 and cdk6, which are critical for progression through the G1 phase of the cell cycle, subsequently the

enzymatic activity of cdks is dependent on specific association with G1 cyclins. So, the cyclin D1/cdk4 complex mediates progression of cell cycle early in G1 phase and inactivates the retinoblastoma protein (pRb), a tumor suppressor by phosphorylation [117,118]. Then, cyclin E/cdk2 complex controls the transition from G1 to phase S by phosphorylation of pRb, a key step in this transition (Fig. 3). Indeed, resveratrol can block the G1/S transition of the cell cycle [39,64,119-122]. Resveratrol is able to act at this point by decreasing the protein expression of cyclin D1, D2, E and the protein expression of cdk 2, 4, 6 and the activities of kinases examined in various cancer cell lines (Fig. 3) [120,123-126]. A loss of cyclin D/cdks or cyclinE/cdks

kinase activities before the restriction point prevents cells from entering S phase and so resveratrol increased the number of cells in G1 phase. Moreover, if resveratrol reduces levels of the cyclin D/cdk4 complex, then it can decrease the activation of cyclin E/cdk2 binding-induced by cyclin D/cdk4 complex. Cyclin-dependent kinase must phosphorylate some substrates whose modification is required for G1 exit, and the retinoblastoma protein is such a target [127]. Indeed, at the restriction point control (R, which represents the point that separates the mitogen-dependent early G1 phase from the mitogen-independent late G1 phase) pRb phosphorylation is triggered by the cyclin D-cdk complex, which in turn releases RB-bound E2F (Fig. 3). So, E2F-DP heterodimers can trigger the expression of various genes essential for S phase progression and DNA synthesis (e.g. cyclin A, E cdc2, dihydrofolate reductase, thymidine kinase, ...). Several reports have shown that resveratrol can decrease the hyperphosphorylated form of pRb with a relative increase in hypophosphorylated pRb [123,128,129]. This response is accompanied by downregulation of protein expression of all five E2F (1-5) family members of transcription factors studied in their heterodimeric partners DP1 and DP2 [123], and in consequence the increase of hypophosphorylated pRb that, in turn, limits with the availability of free E2F [123]. Resveratrol can also block the G1/S transition through an induction of cdk inhibitors (cdki) which can inhibit the active cyclin/cdk complex. Among them, the Cip/Kip family (p21^{Cip1/Waf1}, p27^{Kip1}) may interact with a broad range of cyclin/cdk complexes, as well as the INK4 family (p16^{INK4A} or multiple tumor suppressor 1 gene MTS1, P15^{INK4B} or multiple tumor suppressor gene MTS2). Resveratrol is able to induce cdk inhibitors such as p53-inducible p21^{Cip1/Waf1} involving an G1 arrest by inhibiting the cyclin D1/D2-cdk2, cyclin D1/D2-cdk4, and cyclin E-cdk2 complexes, thereby imposing an artificial checkpoint at the transition G1 S transition of the cell cycle (Fig. 3) [120,128]. At a molecular level, resveratrol up-regulates p53 expression and induces nuclear translocation of p53, leading to the induction of p21 [130-132], but p53 is not the only factor by which resveratrol activates p21. Indeed, resveratrol can up-regulate p21^{Cip1} transcription through a selective up-regulation of the transcription factor Egr-1 [133]. This up-regulation is an Erk1/2-dependent mechanism which induces a binding of Erg-1 *in vitro* and *in vivo* to the consensus sequence of the p21 promoter [133]. p53-inducible p21^{CIP1} and p27^{KIP1} can induce G1 arrest by inhibiting the cyclin D, E and A-cdk complex, while INK4 proteins antagonize only the cyclin D-cdk complex. A single study using acute leukemia cells which were deficient in functional p53 and p16^{INK4} showed that resveratrol induces an arrest in the S phase associated with apoptosis [134]. In parallel, the c-Myc pathway also directly contributes to the G1/S transition by elevating the transcription for cyclin E and cdc25A, which is able to remove the inhibitory phosphates from cdk2 [135]. Resveratrol is able to decrease the protein expression of c-Myc *in vitro* [81], perhaps by a decrease in its transcription *via* an inhibition of E2F, and subsequently the polyphenol could decrease the activation of cyclin/cdk2 complex *via* a low level of c-Myc (Fig. 3). Furthermore, the decrease in cyclin D could be due to competitive action of resveratrol against transcription factors. Indeed, cyclin D1 is a gene that is under the control of AP-1 and NF B, and so resveratrol, by inhibiting the

DNA binding of these transcription factors, can help to decrease the protein expression level of endogenous cyclin D1 [136,137]. Finally, resveratrol is able to reduce the proliferating cell nuclear antigen (PCNA) which is a polymerase accessory protein detected in a cell cycle dependent manner [138]. This series of events result in a G1-phase arrest of the cell cycle, which is an irreversible process that ultimately results in the apoptotic death of cancer cells [120].

ii) Resveratrol and Arrest in S Phase

Once cells enter S phase, cyclin A-cdk2 complex phosphorylates DP-1 and inhibits E2F binding to DNA (Fig. 3). Cyclin E mediates entry into S phase, whereas cyclin A accumulates later during S phase [118]. Unlike other natural compounds such as daidzein and flavone, which inhibit only the cell cycle at G1 phase, various reports show that resveratrol also has a great effect on the S phase with consequent effects on S/G2 transition in various cell lines or in animal models (Table 1) [80,122,125,129,131,134,139-149]. We have shown on adenocarcinoma cell line that resveratrol-induced proliferation arrest is associated with an accumulation of cells in the S phase which is reversible, but a continuous resveratrol treatment blocks the progression of colon cancer cells during the S/G2 transition [141]. Biochemical analysis shows a significant increase of cyclins A and B1 with the accumulation of cdk1 and cdk2, which are also increased in their inactive phosphorylated forms. In fact, cdk1 kinase is known to be a key regulator of the eucaryotic cell cycle and is believed to act in both G1 and G2 phases where the dephosphorylated form is required. In the same case, cdk2 plays an important part throughout the cell cycle where the cdk2 protein expression and its phosphorylation state are regulated with respect to cell cycle phase. Moreover, cdk2 kinase activity has been shown to be required for DNA synthesis [150]. Indeed, it has been shown that the accumulation of the inactive tyrosine 15-phosphorylated cdk1 form is evidence of a cell division cycle arrest preventing the entry into G2/M phases [151,152]. Cyclin A/cdk2 complex plays a key role during S phase progression and cyclin B1 / cdk1 complex controls the cell entry and progression of mitotic phase [M phase] [153,154]. Since our results show that resveratrol provokes a hyperphosphorylation of cdk1 in SW480 cells, one can suggest that resveratrol disrupts the dephosphorylation process of cdk1 leading to the arrest in the S phase (Fig. 3). The same disruption through the cell cycle was observed in the epithelial cell during resveratrol treatment with a hyperphosphorylation of cdk1 [80,129,146], and accumulation of p53 and p21^{WAF1/CIP1} [155], while Surh *et al.* [147] reported that in leukemia HL60 cells, the expression of p21^{WAF1/CIP1}, an inhibitor of cell progression, is not altered by resveratrol treatment. In human melanoma cells, resveratrol induces irreversible S phase arrest and upregulates the expression of cyclins A, E, and B1, concomitant with a decrease in G0/G1 and G2/M phases [156]. Furthermore, consistent with the entry of cells into S phase, there is a dramatic increase in nuclear cdk2 activity associated with both cyclin A and cyclin E [139]. It seems that resveratrol treatment induces a specific response in a tissue-dependent manner and that this polyphenol may act as cell synchronizing agent. The S phase arrest was also shown *in vivo* where resveratrol exhibits anti-tumour activities on murine hepatoma H22 by a mechanism involving an arrest of the cell cycle by decreas-

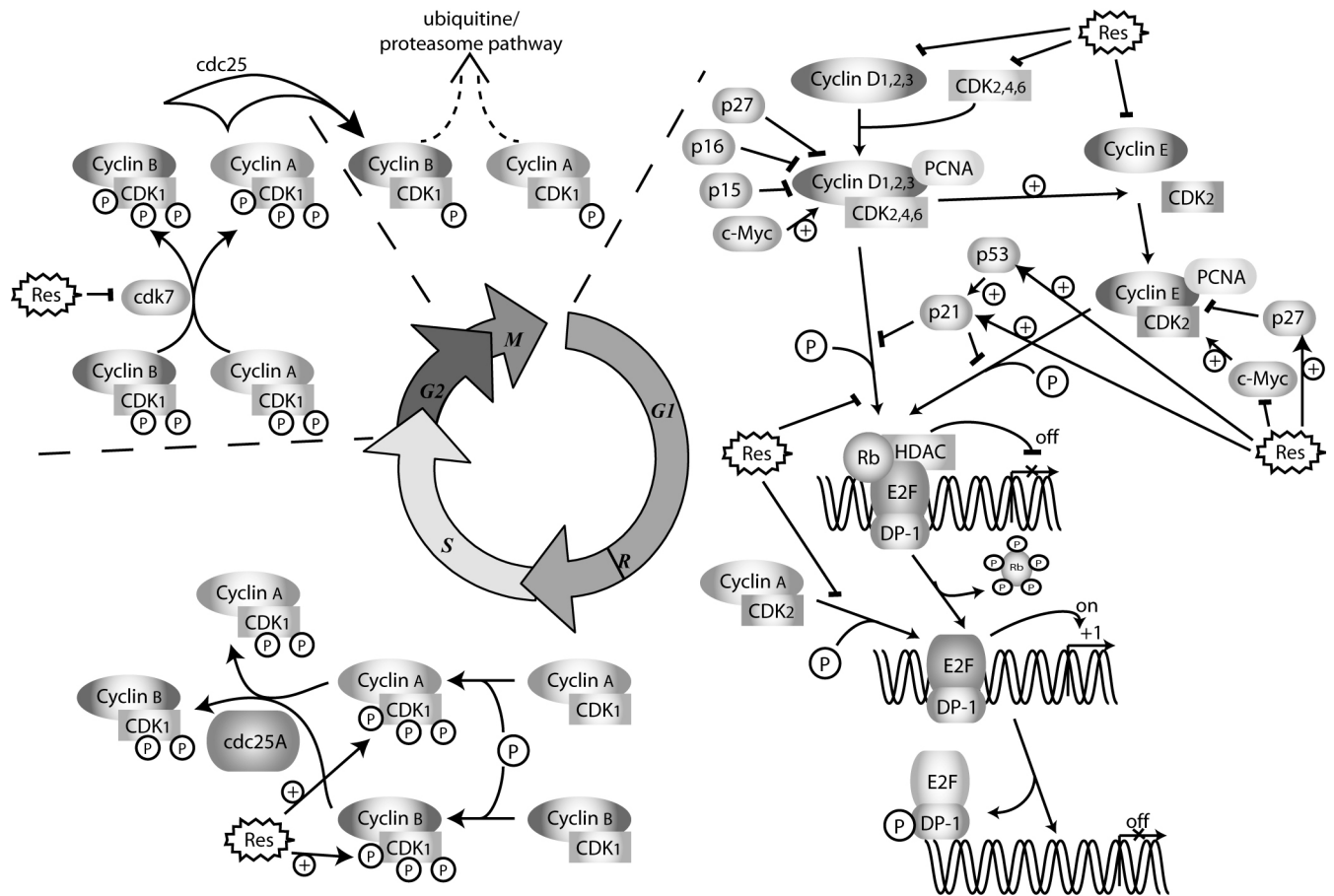


Fig. (3). Resveratrol effects on the key regulators of the cell cycle. According to the cell types, resveratrol is able to block the cell cycle by activation or inhibition of cyclins, cdks, inhibitor of cdks, transcription factors or oncoproteins.

ing the expression of cyclin B1 and cdk1 protein [157]. These results were also shown with an analogue of resveratrol, piceatannol, which induces an accumulation of colorectal cancer cells in the S phase [158]. This arrest is associated with an increase in cyclin A and cyclin E levels, whereas cyclin B1, D1 and cdk4 are downregulated, and the abundance of p27^{Kip1} is also reduced. This S phase arrest is also demonstrated in acute leukemia cells (deficient in functional p53 and p16^{INK4}) in which resveratrol induces an arrest in the S phase associated with apoptosis [134].

Most authors attribute the S phase arrest to an inhibition of ribonucleotide synthase and DNA synthesis. In fact, resveratrol scavenges the essential tyrosyl radical of the small protein of ribonucleotide reductase and, consequently, inhibits deoxyribonucleotide synthesis during the S phase [159,160]. Resveratrol is a much more effective inhibitor than hydroxyurea or hydroxyanisole, the only ribonucleotide reductase tyrosyl radical scavengers used in clinics, or indeed the potent *p*-propoxyphenol [159]. It is also suggested that inhibition of DNA synthesis occurs at the level of DNA polymerase activity, since the recruitment of PCNA and replication protein A (RPA) proteins to DNA replication sites is not affected by resveratrol [161]. More specifically, *in vitro* assays demonstrate that only *trans*-resveratrol significantly inhibits DNA polymerase and [161-163]. Stivala *et al.*

have shown that the inhibition by resveratrol is found to be strictly specific for the B-type DNA polymerases and [161]. Moreover, the structure-activity relationships show that 4'-hydroxyl group in *trans*-conformation of resveratrol (hydroxystyryl moiety) is not the sole determinant for anti-oxidant properties, but acts synergistically with the 3- and 5-OH groups, and that the 4'-hydroxystyryl moiety of *trans*-resveratrol interacts with DNA polymerase [161]. Controversially, we and others have shown that resveratrol reduces viability but not DNA synthesis in cancer cells. Indeed, resveratrol increases the DNA synthesis associated with an accumulation of cells in S phase [141,164]. A similar effect is observed in androgen-sensitive LNCaP cells where resveratrol-induced increase in DNA synthesis is associated with an accumulation of cells in S phase and a concurrent decrease in nuclear p21^{CIP1} and p27^{Kip1} levels [139]. A possible mechanism is that resveratrol causes S phase arrest only when sister chromatid exchange is induced as suggested by Matsuoka *et al.* in chinese hamster lung cell lines [165-167].

iii) Resveratrol and G2/M-Phase Arrest

Cyclin B2 is related mainly to the completion of M phase. This cyclin combines with cdk1 to form MPF which plays an important role in the transition from G2 stage to M stage.

Resveratrol can accumulate various cell lines in the G2 phase of the cell cycle (Table 1) [80,129,164,168]. Biochemical analysis demonstrates that the disruption of G2 phase progression by resveratrol is accompanied by the inactivation of cdk1 and an increase in the tyrosine phosphorylated (inactive) form of cdk1. This reduction of cdk1 activity by resveratrol is mediated through the inhibition of cdk7 kinase activity, while cdc25A phosphatase activity is not affected. In addition, resveratrol-treated cells were shown to have a low level of cdk7 kinase-Thr (161)-phosphorylated cdk1 [168]. *In vitro* and *in vivo* studies show that resveratrol-induced cell cycle arrest in G2/M phase is associated with an accumulation of cyclins A and B [157,164,169,170]

c) Resveratrol Chemoprevention by the Induction of Cell Death

Induction of apoptosis in precancerous or malignant cells is considered to be a promising strategy for chemopreventive or chemotherapeutic purposes. The induction of apoptosis triggered by resveratrol has been observed in various cell types with different pathways. Indeed it has been demonstrated that resveratrol is able to activate cell death by the mitochondrial pathway or by the death receptor pathway. The mitochondrial pathway is used in response to extracellular signals and internal disturbances such as DNA damage.

Major external signals triggering apoptosis are mediated by receptor/ligand interactions (such as CD95 and tumor necrosis factor receptor). The binding of ligand to receptor induces receptor clustering and the formation of death inducing signaling complex (DISC). This complex recruits *via* the adaptor FADD (Fas-associated death domain protein), multiple procaspase-8 molecules resulting in caspase-8 activation and can activate the proteolytic cascade or / and converge on the mitochondrial pathway through the activation of a pro-apoptotic member of the Bcl-2 family (Fig. 4). We and others have shown that resveratrol down-regulates Bcl-2 protein expression [125,147,171-174] and gene expression [175-177], which normally stabilizes the mitochondrial potential of the membrane (ψ_m), and inhibits ROS production. Interestingly, in the CEM-C7H2 T-ALL (acute lymphoblastic leukemia) cell line, which stably overexpressed Bcl-2, resveratrol-induced apoptosis (phosphatidylserine exposure, caspase activation, DNA damage) is inhibited [178]. We have recently show in human colon cancer cells that resveratrol also downregulates Bcl-x_L and Mcl-1 proteins [174]. This observation is also reported in other cell types [125,179,180].

On the other hand, resveratrol could induce apoptosis of tumor cells by modulating pro-apoptotic Bcl-2 family proteins which are known as "BH3-only proteins" behaving as sensors of cellular damage and initiating the agents of death process. Contrary to Bcl-2, resveratrol has been shown to trigger an increase in Bax and Bak protein expression [125,148,174,179-181] and gene expression [175,177,181]. However, a bax-independent pathway to cell death has been identified in a HCT116 colon cancer cell clone in which both bax alleles had been inactivated [182]. The ability of resveratrol to trigger colon cancer cell apoptosis in the absence of Bax could be explained by the functional interchangeability of Bax and Bak. Cells from mice deficient in both Bax and Bak, but not cells deficient in only one of the two, are

almost completely resistant to mitochondria-mediated apoptosis [183]. We have shown that an exposure of adenocarcinoma colon cells to resveratrol induces conformational changes and mitochondrial redistribution of both Bax and Bak, suggesting that the two proteins are involved in resveratrol-induced cell death [174] (Fig. 4). In addition, Bax has been shown to be involved in the chemopreventive effect of resveratrol in animal models of colon carcinogenesis, where Bax expression is enhanced in aberrant crypt foci (AFC) but not in the surrounding mucosa [184]. So, we show that resveratrol-induced apoptosis by this mechanism involves the release of molecules such as cytochrome c, Smac/Diablo contained in the intermembrane space of the mitochondria in the cytosol under the control of Bcl-2 and Bcl-2-related proteins such as Bax (Fig. 4). Cytochrome c, in the cytosol, induces oligomerization of the adapter molecule Apaf-1 to generate a complex, the apoptosome, in which caspase-9 is activated. Active caspase-9 then triggers the catalytic maturation of caspase-3 and other resultant caspases, thus leading to cell death. Resveratrol induces other soluble molecules released from the mitochondria including Smac/Diablo [174,179] that neutralizes caspase inhibitors of the IAP family such as XIAP (Fig. 4) [185,186]. Resveratrol itself is able to inhibit IAP family protein expression such as survivin expression [131,187]. A direct effect on apoptosis by downregulating bcl-2 expression and upregulating bax expression with p53 can occur and activate caspases [188]. It appears that resveratrol can induce an increase in the tumor suppressor gene p53 in various cell types [189-191] and induce its phosphorylation [62,74-76,192,193]. This activation of the transcription factor p53 by resveratrol could contribute to death and cell cycle arrest [53,73,74], but the polyphenol can also induce apoptosis in p53-deficient cells [194,195], indicating that p53 is not an absolute requirement for the cytotoxic effect of the molecule. An initial description of the death pathway triggered by resveratrol in tumor cells involved the up-regulation of Fas-L mRNA and the Fas-L/Fas interaction in an autocrine or paracrine manner [196] but these results were subsequently challenged by several groups in various tumor models [134,178,197-200], based on the observations that i) Fas-L mRNA up-regulation was not confirmed, ii) Abs that prevent the FasL/Fas interaction did not prevent resveratrol-induced apoptosis and iii) cell lines resistant to Fas-mediated apoptosis, e.g. leukemic cell lines, still underwent resveratrol-induced cell death. These arguments do not rule out the possibility of a Fas role in resveratrol induced cell death. Indeed, it has been shown that the death receptor is involved in cytotoxic drug-induced tumor cell apoptosis through a Fas-L-independent, FADD-dependent pathway [201]. Receptor-mediated apoptosis was shown to depend upon prior activation of caspase-8, which was then capable of activating the other later caspases resulting in apoptosis. In addition, the Fas pathway may not be an absolute requirement for resveratrol-induced apoptosis, although it contributes to cell death when functional. The activation of caspase-8 identified in resveratrol-treated adenocarcinoma colon cells could occur earlier in the process at the level of death receptors or later in the process in the caspase cascade to amplify the apoptotic pathway. We have provided a potential explanation for these controversies by showing that resveratrol does not increase the expression of Fas and FasL at the surface of tumor cells but does induce a redistribution

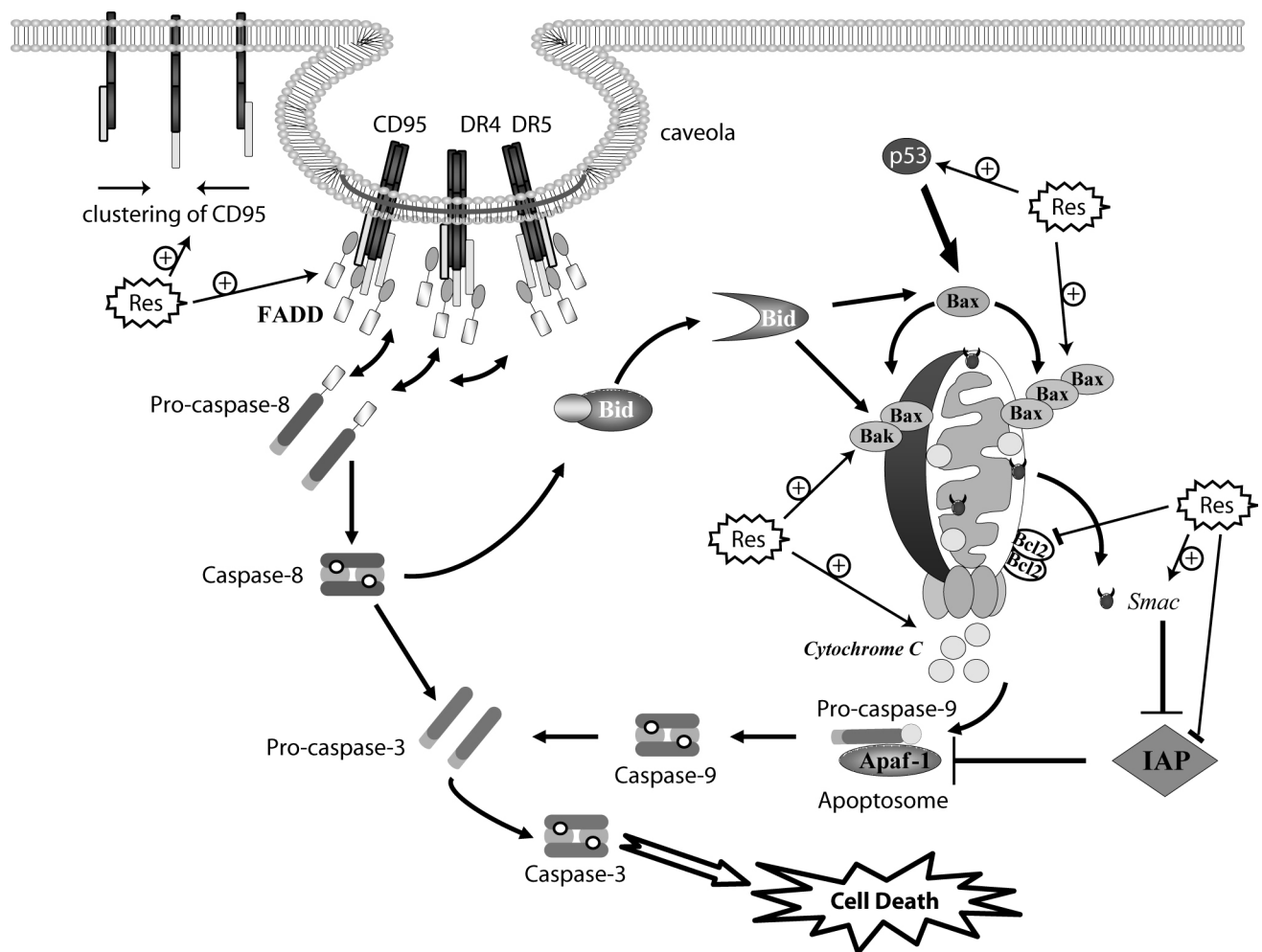


Fig. (4). Resveratrol effects on the apoptosis pathways. Resveratrol down-regulates Bcl-2 and IAP protein expression and induces an increase of Bax/Bak and their relocalization to the mitochondria. These events contribute to the release into the cytosol of cytochrome c and Smac. Furthermore, resveratrol induces the clustering of death receptors into lipid microdomain (rafts) which formed caveola. Then, resveratrol induces the DISC formation leading the caspase cascade activation.

of Fas in the raft domains of the plasma membrane [174]. These lipid microdomains result from the preferential packing of complex sphingolipids and cholesterol in ordered plasma membrane structures and contain a variety of lipid-anchored and transmembrane proteins. Rafts play an important role in clustering or aggregating surface receptors, signaling enzymes and adaptor molecules into membrane complexes at specific sites and were shown to be essential for initiating signaling from a number of receptors. It appears that resveratrol treatment changes the homogeneous distribution of the protein existing in untreated colon cancer cells into a more clustered distribution. In addition, resveratrol induces a redistribution of Fas, together with FADD and procaspase-8, in the fractions enriched in cholesterol and sphingolipids (Fig. 4) [174]. The mechanisms trapping receptor molecules in membrane rafts have yet to be characterized. Selective clustering of Fas was suggested to involve the acid sphingomyelinase-induced release of ceramide in lymphocytes or fibroblasts [202]. Other hypotheses include hydrophobic modifications of the receptor, interaction with a binding partner that itself associates with raft lipids, or in-

creased affinity induced by initial clustering of Fas. Whatever these mechanisms, resveratrol-induced redistribution of Fas in the rafts could contribute to the formation of the death-inducing signaling complex (DISC) observed in colon cancer cells treated with the polyphenol. The involvement of this pathway is reinforced by the fact that resveratrol-induced apoptosis is prevented by transient transfection with a dominant negative mutant form of FADD, E8 or MC159 viral proteins that interfere with DISC function [174]. We have also shown that this signaling complex contributes to Bax and Bak conformational changes, caspase-3 activation and apoptosis in resveratrol-treated cancer cells. In another study, we have shown that resveratrol can also redistribute other death receptors such as DR4 and DR5 and form a functional DISC inducing apoptosis [203].

The antipromotional activity of resveratrol can also be attributed to its ability to enhance the Gap Junctional Intracellular Communication (GJIC) in cells exposed to tumor promoters such as TPA [204]. Indeed, GJIC is important for normal cell growth and its suppression can lead to transformation and subsequent tumor promotion

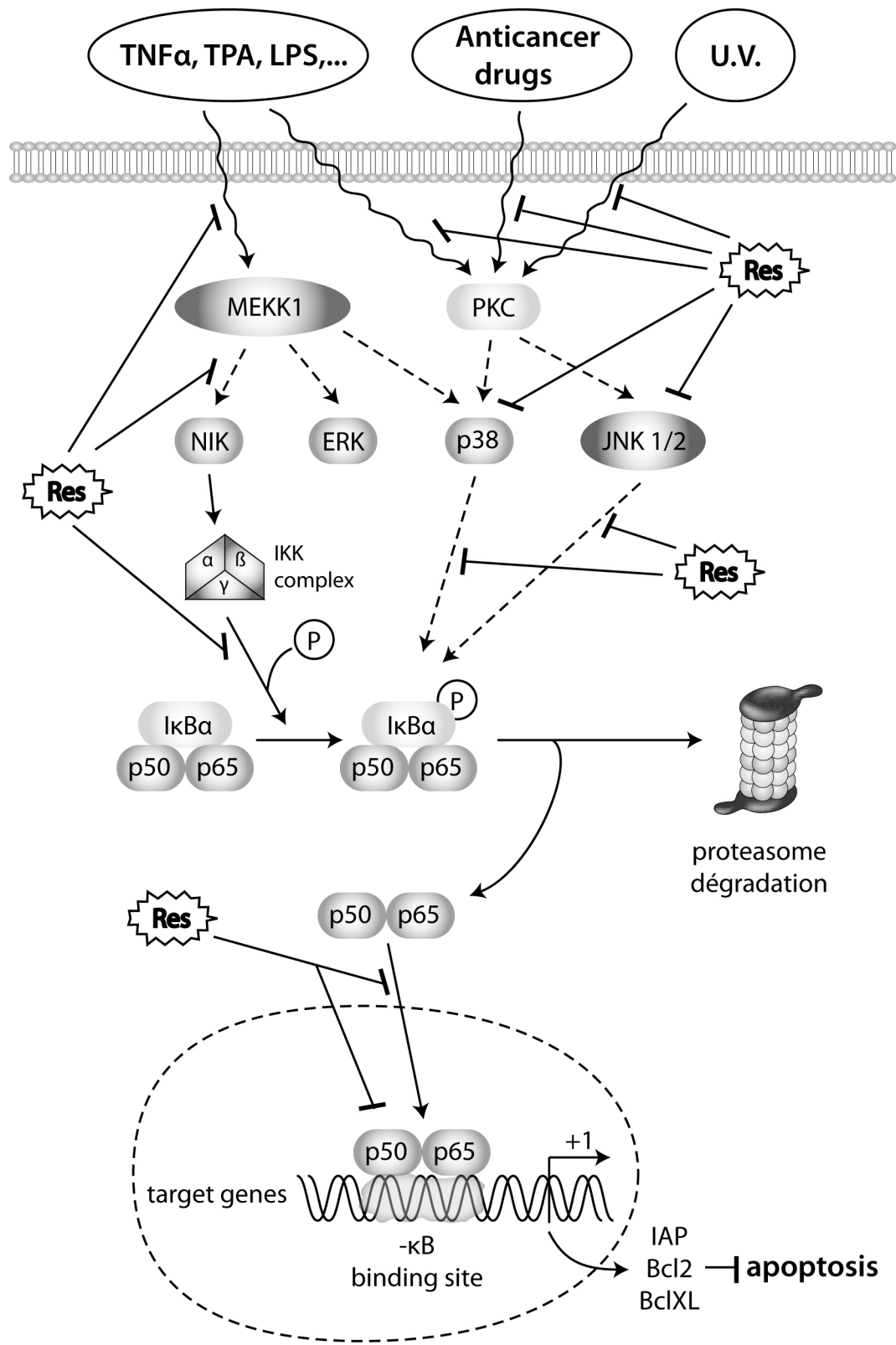


Fig. (5). Resveratrol effects on the kinase cascade. Resveratrol is able to block stimuli-induced kinases such as PKC, MEK, and can also block the activation of MAPK kinases such as p38kinase, JNK or ERK1/2. Moreover resveratrol prevents the phosphorylation of NF B complex and the translocation of p50/p65 subunits to the nucleus, and blocks their DNA binding site. Consequently, the expression of various genes is downregulated.

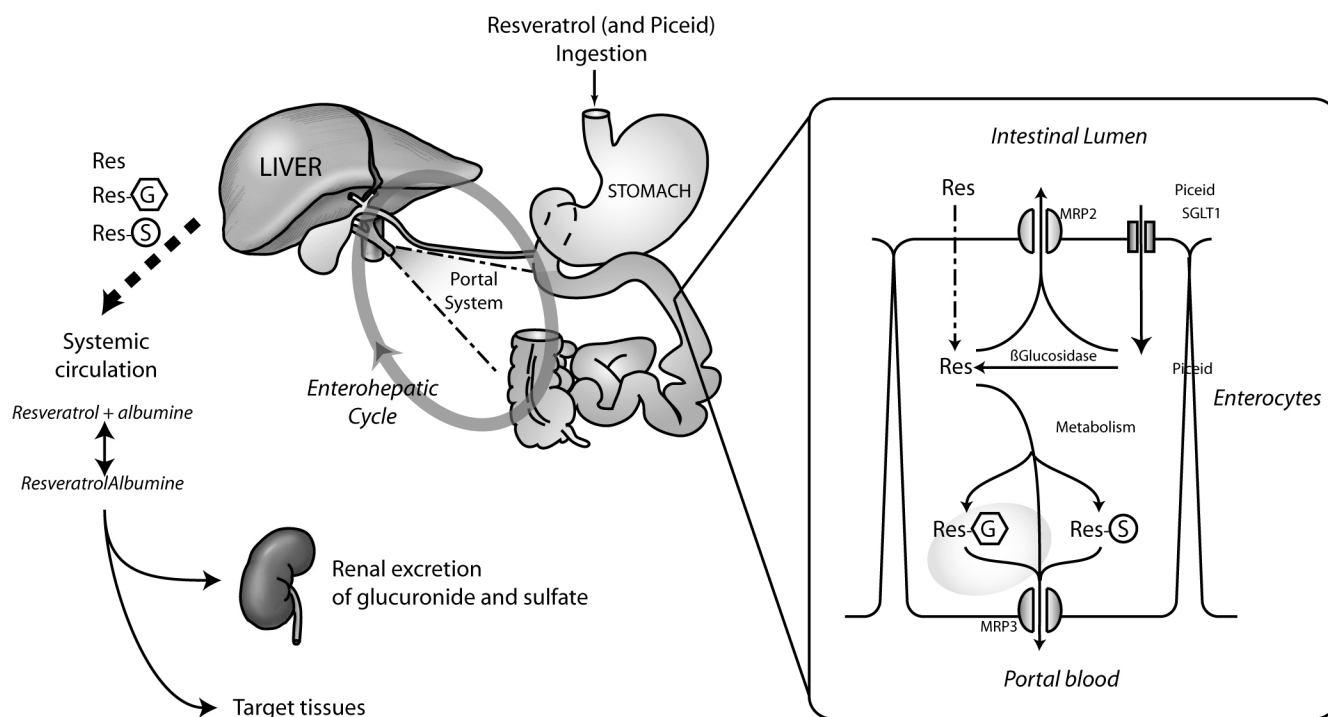


Fig. (6). Distribution and metabolism of dietary resveratrol.

3. Resveratrol and Progression / Invasion

Finally, resveratrol can act on the third step of carcinogenesis, the progression that which is associated with the evolution of the initiated cells into a biologically malignant cell population. In this stage, a portion of the benign tumor cells may be converted into malignant forms leading to a true cancer (Fig. 2). At this stage, tumor progression is certainly too advanced for chemopreventive intervention but not for a chemotherapeutic intervention. During tumor progression, resveratrol, as previously described, can act as an antiproliferative agent by blocking cell cycle progression and inducing apoptosis of cancer cells. The polyphenol can also act on events more specific of the progression / invasion step. In this final stage, invasion, can break away and start new clones of growth distant from the original site of development of the tumor. It was reported that resveratrol showed a greater anti-proliferative effect on highly invasive breast carcinoma cells (MDA-MB-435) than on minimally invasive cells (MCF-7) [171]. Recent studies have shown the involvement of arachidonic acid metabolites in tumor cell invasion and metastasis [205,206]. Since resveratrol is a lipoxygenase [85] and cyclooxygenase inhibitor, the possibility that resveratrol or its metabolite(s) inhibit the invasion of rat ascites hepatoma cells by suppressing lipoxygenase and/or cyclooxygenase activity cannot be ruled out [207].

a) Resveratrol Chemoprevention by Inhibition of Nitric Oxide

The role of nitric oxide (NO) in tumoral progression and metastasis has recently been evaluated in mammary tumor models of mice [208]. In endothelial tumoral cells, endothelial NO synthase (eNOS) promote tumoral growth and me-

tastasis by various mechanisms such as the stimulation of tumoral cell migration, invasion, and angiogenesis. For example, it has been shown that the increase in inducible NO synthase and in eNOS was correlated with tumoral growth and vascular invasion in human colorectal cancer [209]. Resveratrol inhibits the *de novo* formation of inducible nitric oxide synthase (iNOS) in mouse macrophages stimulated with lipopolysaccharide, without affecting COX-2 expression [97]. So, the polyphenol is able to inhibit NO generation in activated macrophages by reducing the amount of cytosolic iNOS protein and by inhibiting the activation of NF κ B induced by LPS [210]. This hydroxystilbene is able to reduce the nuclear content of NF κ B subunits, the nuclear translocation of the p65 subunit of NF κ B, and inhibits the NF κ B phosphorylation and degradation [70,210,211]. So, by disturbing the nuclear factors (NF κ B, AP-1, GATA, ...), resveratrol affects the expression of the iNOS gene which is partly controlled by NF κ B [212]. So, through a negative regulation of NF κ B binding activity *via* a blockage of I κ B degradation, resveratrol can reduce the abnormal concentrations of NO (and its derivatives such as peroxynitrite) which contribute to inflammation and angiogenesis.

b) Resveratrol Chemoprevention by Inhibition of Angiogenesis

Angiogenesis provides a gateway for tumor cells to enter the circulation and, in the reverse direction, for leukocytes to infiltrate the tumor and provide proteolytic enzymes and chemokines, which facilitate the migration and invasion of tumor cells [213]. This phenomenon occurs through the invasion of endothelial cells from existing vessels in response to multiple extracellular signals such as polyamines, vascular endothelial growth factor (VEGF) and fibroblast growth

factor (FGF-1, -2). It seems that the acquisition of angiogenic properties can involve oncogenes and tumor suppressor genes. As we have seen previously, resveratrol is able to inhibit polyamine synthesis and increase their catabolism (see § 2b). So, resveratrol can decrease the blood-vessel development (angiogenesis) occurring in response to damage to normal tissues or to tumor growth through the inhibition of the necessary polyamines [214,215]. Furthermore, various studies have shown that the fall in polyamine levels is associated with a decreased expression of genes affecting tumor invasion and metastasis such as COX, spermidine/spermine N-acetyltransferase (SSAT), ODC [216,217]. So, resveratrol can act on the expression of these genes by the modulation of polyamine levels.

Resveratrol can act on angiogenesis through an inhibition of matrix metalloproteinase, (MMP-9), urokinase-type plasminogen activator and adhesion molecules [218]. Indeed, resveratrol was able to inhibit hypoxia-inducible factor 1 alpha (HIF-1 α) and VEGF expression in human ovarian cancer cells [219]. At a molecular level, resveratrol inhibits these factors through an inhibition of AKT and MAPK activation, that play a partial role in the down regulation of HIF-1 α expression. Furthermore, the polyphenol inhibits insulin-like growth factor 1-induced HIF-1 α expression through the inhibition of translational regulators such as p70 ribosomal protein S6 kinase (p70(S6K)), eukaryotic initiation factor 4E-binding protein 1, and eukaryotic initiation factor 4E [219]. Resveratrol is also able to induce HIF-1 α protein degradation through the proteasome pathway. Concerning VEGF, this inhibition by resveratrol is described in various reports [176,219-221]. In fact, resveratrol abrogates VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin and its complex partner, β -catenin [222]. Moreover, resveratrol strongly inhibits VEGF-induced endogenous Src kinase activation. Again, transfection with v-Src, an active form of Src, could reverse resveratrol inhibition of VE-cadherin tyrosine phosphorylation and EC tube formation [222]. One hypothesis is that resveratrol inhibition of VEGF-induced angiogenesis is mediated by disruption of ROS-dependent Src kinase activation and the subsequent VE-cadherin tyrosine phosphorylation. Resveratrol may also block VEGF and FGF-receptor-mediated angiogenic responses. It appears that oral administration of resveratrol delays angiogenesis-dependent wound healing in mice [223]. Its anti-angiogenic mechanism involves direct inhibition of capillary endothelial cell growth *via* the suppression of the phosphorylation of MAPK. This pathway appears to be common to both VEGF- and FGF-2-induced angiogenesis [223].

Efficient tumor invasion also requires partial degradation of the extracellular matrix (ECM) at the invasion front. The matrix metalloproteinases (MMPs) are the main proteases involved in remodeling the ECM contributing to invasion and metastasis, as well as tumor angiogenesis [224]. Concerning the human MMPs, the expression levels of gelatinase-A (MMP-2) and gelatinase-B (MMP-9) are associated with tumor metastasis for various human cancers [225,226]. Resveratrol is able to directly inhibit the gelatinolytic activities of MMP-2 in various cell types [227,228]. Resveratrol is also able to suppress DMBA-induced MMP-9 expression by inhibiting NF- κ B DNA binding [57,65]. In

fact, resveratrol is able to inhibit MMP-9 activity, this inhibition MMP-9 expression is achieved *via* reduced PKC-activity as well as diminished JNK activation [65]. So, resveratrol treatment also inhibits endothelial cell attachment to basal membrane components fibronectin and laminin, and displays a similar effect on cell chemotaxis [228]. Moreover, resveratrol is able to inhibit the adhesion molecule expression such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-2 (VCAM-2) [229-231]

c) Resveratrol Chemoprevention by Activation of Kinase Cascade

We have previously shown that resveratrol can act as a chemopreventive agent by inhibition of stimuli-induced kinase cascades, but the polyphenol can also activate MAPK pathways and subsequently induce the activation of the cell death pathway. Indeed, resveratrol is shown to activate extracellular-signal-regulated protein kinases (ERKs), p38 kinase and c-Jun NH2-terminal kinases (JNKs) and their phosphorylation [72-76]. By their resveratrol-induced phosphorylation, MAPK plays a critical role in the stabilization, up-regulation and functional activation of p53 [74]. Moreover, resveratrol acts *via* a Ras-MAPK kinase-MAPK signal transduction pathway to increase p53 expression, serine phosphorylation of p53, and p53-dependent apoptosis in papillary thyroid carcinoma (PTC) and two follicular thyroid carcinoma (FTC) cell lines [76]. The polyphenol causes ERK1/2 activation and nuclear translocation of ERK1/2 [62,76]. In fact, resveratrol induces activation of ERKs and p38 kinase and the phosphorylation of p53 at serine. She *et al.* have shown that the stable expression of a dominant negative mutant of JNK1 or the disruption of the *jnk1* or *jnk2* gene markedly inhibited resveratrol-induced p53-dependent transcription activation and induction of apoptosis [73]. A recent report shows that resveratrol activates MAPK signaling through estrogen receptors alpha and beta in endothelial cells [232]. Interestingly, at nanomolar level, resveratrol was able to activate MAPK in an estrogen receptor- (ER), MEK-, MMP-, Src-, and EGF-R-dependent manner in endothelial cells [232]. It might be that oral ingestion of low concentrations of resveratrol could result in transient serum levels leading to the activation of membrane-initiated ER signaling that would activate MAPK. Another pathway inducing apoptosis could involve the MAPK pathway such as ASK1 (apoptosis signal-regulating kinase-1)/JNK. Indeed, resveratrol induces apoptosis through the Cdc42/apoptosis signal-regulating kinase 1A/c-Jun N-terminal kinase/FasL signaling cascade in human promyelocytic leukemia cell lines [233]. Resveratrol also activates the small GTP-binding protein Cdc42, rather than other members such as RhoA or Rac1 [233].

C) RESVERATROL, AN ADJUVANT FOR CHEMOSENSITIZATION AND RADIOSENSITIZATION

Despite aggressive therapies, resistance of many tumors to established treatment procedures still constitutes a major problem in cancer therapy. Recent evidence suggests that the use of resveratrol in combination with drugs, ionizing radiation or cytokines, can be effectively used for the sensitization to apoptosis. It appears that resveratrol can

sensitize to various cytotoxic agents such as cyclosporin, paclitaxel, 5-fluorouracil. For example, the combination resveratrol / cyclosporin A is a synergy reducing the proliferation and transformation of human peripheral blood T lymphocytes (hPBTCs) and enhancing immune suppression [234]. Paclitaxel is also an essential chemotherapeutic agent in lung cancer treatment, and a pretreatment with resveratrol amplifies the antiproliferative and pro-apoptotic effects of this drug, but it is not the case with a simultaneous exposure [235]. In fact, a pretreatment with resveratrol induces p21^{WAF1} expression suggesting a possible arrest of cell cycle favouring the effect of paclitaxel action. This could be the case of the combination with 5-fluorouracil (5-Fu) which is a classic drug used in colorectal and hepatoma chemotherapy. Indeed, it was reported that resveratrol can exert synergic effect with this drug to inhibit hepatocarcinoma cell proliferation by the induction of apoptosis [236,237].

A pretreatment with resveratrol prior to ionizing radiation (IR) exposure of resveratrol radiosensitizes human cervical tumor cell lines enhances tumor cell killing by IR in a dose-dependent manner [238]. In fact, pretreatment with resveratrol alters both cell cycle progression in the S phase, blocking cell division and the cytotoxic response to IR in cervical tumor cell lines. This explains the recently reported ability of resveratrol to enhance radiation-induced apoptosis of cancer cell lines such as HeLa (cervix carcinoma), K-5562 (chronic myeloid leukemia) and IM-9 (multiple myeloma); this occurs only at high concentrations of resveratrol [239].

Concerning cytokines, we and others have shown that resveratrol is able to sensitize to TRAIL (tumor necrosis factor-related apoptosis-inducing ligand)-induced apoptosis in cancer cells [131,203]. In neuroblastoma cells, treatment with resveratrol sensitizes these cells to TRAIL-induced apoptosis in the absence of a functional p53 pathway [131]. This sensitization involves a cell cycle arrest-mediated survivin depletion and an upregulation of p21 [131]. In human colon cancer cells that are resistant to the cytotoxic effect of resveratrol, we have shown that resveratrol sensitizes these tumor cells to TNF, anti-CD95 antibodies and TRAIL-mediated apoptosis and activates a caspase-dependent death pathway that escapes Bcl-2-mediated expression [203]. It appears that resveratrol pretreatment facilitates the formation of a functional DISC at plasma level. The cholesterol sequestering agent nystatin prevents resveratrol-induced death receptor redistribution and cell sensitization to death receptor stimulation, suggesting that resveratrol-induced redistribution of death receptors in lipid rafts is an essential step in its sensitizing effect expression [203].

D) BIOAVAILABILITY OF RESVERATROL

An important cause of failure in cancer therapies is due to a defect of drug accumulation in cancer cells. Indeed, the action of chemopreventive or chemotherapeutic agents can be nullified by a failure of their absorption, distribution, metabolism or an increase in their excretion. Moreover, various chemotherapeutic drugs used in clinical treatments cause hematological- and hepato-nephro-toxicities. Several reports have studied the absorption of resveratrol. Resveratrol intestinal absorption was first demonstrated by

Andlauer *et al.* in *ex vivo* rat intestine [240]. The high extent of resveratrol bioabsorption was shown in rat where 50% to 75% of resveratrol was absorbed [241]. Kaldas *et al.* also reported a fast transport of resveratrol across the cell layer of intestine Caco-2 cell line [242]. Recently, Henry *et al.* (2005) showed that apical transport of resveratrol follows a passive mechanism, while the glycoside (*trans*-piceid) is taken up by the active glucose carrier SGLT1. An apical active efflux of resveratrol through MRP2 protein cannot be excluded [243].

The degree of resveratrol absorption can be evaluated by the plasmatic concentrations of total resveratrol (free + metabolites) after oral consumption. Several studies report the levels of resveratrol recovered in plasma in rodents and humans [241]. After administration to rats of dosages mimicking a human moderated consumption of corne rich in resveratrol, the resveratrol in plasma shows a peak around one hour after ingestion [244]. In humans, Goldberg *et al.* (2003) detect the peak of resveratrol 30 min after ingestion, corresponding to a higher plasmatic concentration than with other polyphenols ((+) catechin, quercetin), and consequently to a better absorption [245]. The oral ingestion of resveratrol by human volunteers led to a concentration of 2 μ M of total resveratrol and a plasma half-life ranging from 6.5 to 14.9 h; based on the urinary excretion data, the absorption of resveratrol appeared to be at least 70% [246].

However, despite an efficient absorption, the plasmatic levels of free resveratrol (aglycone) remain very low. This discrepancy underlines the importance of resveratrol metabolism. For example, after oral administration of 20 mg/kg of *trans*-resveratrol to rabbits, rats or mice, the highest concentration in plasma (2-3 μ M in mice and 1 μ M in rabbits or rats) is found within the first 5 min and decreases to less than 0.1 μ M at 60 min. In rats treated by gavage with high dosages (300, 1000 and 3000 mg/kg), plasmatic concentrations of resveratrol reach 2.5, 4.3 and 12 μ M respectively after one hour. In this case, the effective concentrations are compatible with those required for the *in vitro* inhibiting effect of resveratrol [247]. In humans, after a resveratrol oral dose of 25 mg (20x higher than the amount resulting from a daily intake of a moderate quantity of red wine) free resveratrol is only present in plasma in minute amounts, i.e. 35 nM [245] or 22 nM [246], while the metabolites are present in high amounts. In this last study, the excretion of resveratrol and derivatives was found to occur essentially in urine.

Several metabolic transformations are relevant, the mechanism of several agents. Resveratrol may be oxidized at the level of a phenolic ring. The conversion of resveratrol by cytochrome P450 was demonstrated only by *in vitro* studies. Resveratrol is hydroxylated by a microsomal preparation, rich in CYP1B1, in piceatannol (3,4,3',5'-tetrahydroxystilbene) [248]. CYP1A2 was shown to be involved in the hydroxylation of resveratrol in human liver microsomes, in piceatannol, another tetrahydroxystilbene [249]. Resveratrol may be reduced at the level of its double-bond. Walle (2004) reported such a reaction. Dihydroresveratrol probably results from the hydrogenation of the aliphatic resveratrol double bond by the intestinal bacterial microflora [246].

Andlauer *et al.* (2000) showed in rat intestine model that 17% of resveratrol is transformed into glucuronide and only 0.3% into sulfate [240]. These metabolites are also found in intestine lumen (11% of glucuronides and 3% of sulfates). Unexpectedly, in humans, sulfate-resveratrol conjugate is the main plasmatic metabolite a few hours after administration. It is ejected from the apical side of the cell in the presence of low resveratrol concentrations. The resveratrol metabolites detected by HPLC are the following: two resveratrol monoglucuronide isomers, dihydroresveratrol monoglucuronide, resveratrol monosulfate, dihydroresveratrol monosulfate [246]. Dihydroresveratrol probably results from a catalytic saturation of the resveratrol double bond by the intestinal bacterial microflora. Conjugation also occurs in liver. Resveratrol is sulfated in the human liver [250] and also glucuronidated [251]. Aumont *et al.* (2001) showed using liver microsomes that the glucuronidation of resveratrol is regioselective and stereospecific, leading to the formation of two glucuronides (3-O- and 4'-O-glucuronides) [252]. The reaction is catalyzed by UDP-glucuronosyltransferases of the family 1A. Two major metabolites have been characterized by LC-MS on human hepatocytes: trans-resveratrol 3-O-glucuronide and trans resveratrol-3-sulfate [253].

After short-term or prolonged administration of red wine to rats, resveratrol was found in heart, liver and kidney [254]. Vitrac *et al.* have detected radiolabeled ^{14}C -resveratrol in different organs of mice [255]. They have reported significant resveratrol concentrations in the gut tract (stomach, intestine), in detoxifying organs (liver, kidney) and in urine. Our own studies have shown that resveratrol uptake is very fast in isolated human hepatocytes and in hepatoblastoma HepG2. Moreover, the absorption by hepatic cell is due to both passive diffusion and an active process [256]. Albumin is an important carrier for resveratrol and is very likely to play an important role in its distribution to the tissues [257].

Despite the small number of *in vivo* studies, all of them come to the same conclusion: resveratrol is efficiently absorbed by the organism, but unfortunately has a low level of bioavailability, glucuronidation and sulfation being limiting factors. Nevertheless, some elements may increase resveratrol bioavailability. Interestingly, Walle *et al.* (2004) still detect a blood peak of 1.3 μM after 6 hours, indicating an entero-hepatic recirculation of metabolites reabsorbed after intestinal hydrolysis [246]. Conversely, Yu *et al.* (2002) consider that oral absorption of resveratrol (and not gavage) may increase transiently free resveratrol concentration in the blood *via* buccal mucosa absorption [253]. Moreover, it is probable that tissue sulfatases and glucuronidases can hydrolyze conjugated resveratrol and lead to higher local tissue concentrations of aglycone.

As underlined by Yu *et al.* (2002), *in vitro* studies using unconjugated resveratrol only need to be completed by experiments with metabolites of resveratrol [253]. While bioavailability is better understood in the gut tract and related organs, *in vitro* and *in vivo* studies on lung and breast cell/tissue must consider resveratrol metabolism and tissue bioabsorption. For human protocols in cancer treatments, it is important to take into account resveratrol metabolites.

Moreover, some derivatives of resveratrol may present a better bioavailability. For example, 3,4,5,4'-tetrahydroxystilbene exhibits superior availability compared to resveratrol with good biological effects [258]. Conversely, the glycoside forms of resveratrol are absorbed less well than aglycone [259].

E) CONCLUSION

There is compelling evidence that resveratrol can act on the carcinogenesis process by affecting the three phases: tumor initiation, promotion and progression phases. It appears that resveratrol can prevent metabolic activation, ROS production, adduct formation and stimulate metabolic inactivation. Resveratrol is also able to act against the chemical carcinogens and other various stimuli by several mechanisms such as activation of apoptosis, arrest of the cell cycle or inhibition of kinase pathways. Resveratrol is able to suppress the final steps of carcinogenesis, namely angiogenesis and metastasis. Ideally chemopreventive agents act at safe doses effectively affect the carcinogenic process without toxicity. Interestingly, resveratrol does not present any cytotoxicity in animal models. A recent study shows that resveratrol at nanomolar concentrations is able to act on the estrogen receptors and MAPK pathways [232]. Moreover, concentrations of resveratrol and / or metabolite(s) in blood seem to be sufficient for anti-invasive activity. It is likely that most of the resveratrol might have been metabolized into compound(s) which preserve anti-oxidative activity but lose anti-proliferative activity. It would be necessary to study the effects of the different metabolites on several cancer types. In fact, the highly polar conjugates are generally inactive and are rapidly excreted in the urine and feces. Enterohepatic recirculation, which releases the parent drug into the systemic circulation, may be associated with a delayed elimination of the drug from the body and a prolongation of its effect. By its binding to plasmatic proteins, the effect of resveratrol could be prolonged. Another property of the polyphenol is drug-chemosensitization. Firstly, resveratrol, through its effect on drug biotransformation enzymes, could lead to an enhancement of the level of therapeutic drugs and a prolongation of their pharmacological effects, but could also lead to an increase in their toxicity. It appears that low doses of resveratrol can sensitize to low doses of cytotoxic drugs and so provide a novel strategy to enhance the efficacy of anticancer therapy in various human cancers. This sensitization can involve i) an arrest of the cell cycle such as in S phase where the cancer cells are sensitized to antimetabolite drugs (5-fluorouracil, doxorubicin...); ii) a sensitization to stimuli-induced apoptosis; iii) an inhibition of P450 3A4 which contributes to the poor oral bioavailability of many drugs. Furthermore, several groups develop other forms of resveratrol such as oxyresveratrol which is a more effective scavenger than resveratrol, but a less effective inhibitor of iNOS activity [260]. Other groups have developed a methylated derivative *trans*-3,5,4'-trimethoxystilbene which is able to inhibit tubulin polymerization and induce apoptosis at very low concentrations, but it seems that compounds are more toxic than resveratrol [261,262]. Thanks to all these properties, resveratrol seems to be a good candidate in chemopreventive

or in chemotherapeutic strategies and could be a potential weapon for new therapeutic strategies.

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ABBREVIATIONS

| | |
|------|---|
| COX | = Cyclooxygenase |
| DR | = Death receptor |
| ERK | = Extracellular signal-regulated protein kinase |
| iNOS | = Inducible nitric oxide synthase |
| JNK | = c-Jun N-terminal kinase |
| MPAK | = Mitogen-activated protein kinases |
| NF B | = Nuclear factor kappa B |
| ODC | = Ornithine decarboxylase |
| PHA | = Polycyclic aromatic hydrocarbons |
| ROS | = Reactive oxygen species. |

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