Review

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Anti-tumor effects and cellular mechanisms of resveratrol

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Summary **Resveratrol (3, 5, 4'-trihydroxystilbene) is a phytoalexin contained in a variety of plants, such as grapes, berries and especially in the dried roots of *Polygonum cuspidatum* Sieb. et Zucc. It has been shown to exhibit anti-oxidative and anti-inflammation activity, and to reverse the effects of aging. Its ability to suppress cell proliferation, induce apoptosis and suppress the metastasis and invasion in a number of cell lines has prompted a large interest from people for its use as an anti-tumor component. In this review, evidence of resveratrol's anti-tumor effects and molecular mechanisms are recapitulated. First, we present the anti-apoptosis, anti-invasion/metastasis and anti-inflammation effect of resveratrol; second, the main signaling pathways involved in these activities are described and summarized with the studies of different tumors involved. Resveratrol not only induces apoptosis of tumor cells through intrinsic/extrinsic pathways and cell cycle arrest, but also inhibits the invasion and metastasis abilities of tumors *via* modulating collagen degradation-related molecular targets. Altogether, the present findings suggest the anti-tumor potential of resveratrol against various types of cancers.**

Keywords: Resveratrol, anti-tumor, apoptosis, invasion, metastasis, molecular mechanism

1. Introduction

Resveratrol (3, 5, 4' -trihydroxystilbene) is a phytoalexin contained in a variety of plants, such as grapes, peanuts, berries and especially in the dried roots of a traditional Chinese medicine *Polygonum cuspidatum* Sieb. et Zucc (*1,2*). It exists as two geometric isomers: *cis*-(Z) and *trans*-(E) (*3*), and the *trans*-form can undergo isomerization to the *cis*- form when exposed to ultraviolet irradiation (*4*).

Resveratrol has a protective effect in response to stress, injury, ultraviolet irradiation and fungal infection (*1,2*). Previous studies have demonstrated that resveratrol has a number of biological activities and medicinal uses. It has been shown to exhibit anti-oxidative and anti-inflammation activity, and to reverse the effects of aging in rats (*5*). It also has a cardio-protective effect (*6*) that regular moderate consumption of red wine confers less risk of cardiovascular diseases due to its relatively high resveratrol concentration (0.1-14.3 mg/L), widely known as the "French Paradox" (*7*). There are also other effects, which include phytoestrogen activity (*8*), neuro-protective activity (*9*), and antidepressant activity (*10*).

Importantly, as a natural compound, resveratrol has been highly studied for not only the preventive effect for the diseases mentioned above, but also the anti-tumor effect against various cancers. Its ability to suppress cell proliferation, induce apoptosis and suppress metastasis and invasion in a number of cell lines makes resveratrol a natural weapon in the war against cancer (*11*). Cancer is a multistep disease characterized by uncontrolled cell growth and acquisition of metastatic properties (*12*). In this process, the activation of oncogenes and/or the inactivation of tumor suppressor genes lead to cell cycle arrest and apoptotic pathway suppression. Besides, malignant tumors gain metastasis and invasion ability due to the up-regulation of the pro-metastasis genes and pathways, such as Metalloproteinases (MMPs), and down-regulation of anti-metastasis genes, such as phosphatase and tensin homolog deleted on chromosome ten (PTEN). Cancer cells are known to

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have alterations in multiple cellular signaling pathways, and because of the complex communications between these signaling networks, cure of most human cancers remains a great challenge (*11*). The multiple-target regulating effect of resveratrol mentioned above is the molecular basis for its anti-tumor potential.

The health threat brought by cancer is becoming more severe. Cancer is the second leading cause of death after cardiovascular disorders (*11*), and according to World Health Organization (WHO), cancers figure among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012. Among various kinds of tumor types, the most common causes of cancer death are cancers of: lung, liver, stomach, colorectal, breast and esophageal cancer, and the number of new cancer cases is expected to rise by about 70% over the next two decades.

In this situation, the use of natural compounds of plants in preventing, suppressing or even curing the initiation and progression of malignancy is gaining attention rapidly. Not only because they can influence the processes underlying all three stages of carcinogenesis involving tumor initiation, promotion and progression and suppress angiogenesis and metastasis through regulating different molecular targets (*13*), but also due to their relatively low toxicity (*14*) that are frequently found in the therapy of surgery and chemotherapy. Resveratrol, as one of the most studied dietary compounds, has been demonstrated to interact with molecular targets affecting (anti-) apoptosis (P53, Bax/Bcl-2, Survivin; Caspase-9, 8, 7, 3, PARP), cell cycle (P21, Cyclins, cdks), protein kinases (MAPK, PI3K/AKT, JAK, Wnt), transcription factors (NF-κB, AP-1, Nrf-2), metastasis and invasion (MMP-2, 7, 9, VEGF), and so on (*13*). We discuss the anti-tumor effect and molecular mechanisms (Figure 1) of resveratrol against several cancers mentioned above with the most cancer deaths and both *in vitro* and *in vivo* studies (Table 1), advocating that resveratrol holds enormous potential as an anti-tumor component.

2. Effects of resveratrol on cell proliferation and apoptosis

Apoptosis is a cell death mechanism that may be prompted by several molecular pathways, among which the intrinsic and extrinsic (also known as "death receptor pathway") pathways are the best known (*17*). As it is recognized that a genetically controlled program governed commitment to and execution of apoptosis in a wide range of multicellular organisms, research in understanding the genes and biochemical events responsible for the process of apoptosis have been greatly enhanced (*16*). The discovery of the apoptotic program and the molecular pathways of it have led to development of specific cell-death-targeting therapies, which would allow the development of specific approaches to influence cells by small molecular or protein-based agents to target the apoptotic signaling pathway (*15*) to cure various related diseases including tumors. Resveratrol, as previous studies revealed, is generally a pro-apoptotic agent that could promote the apoptosis process in series of cancers. Here, we separate this part according to several different pathways involved in resveratrol's pro-apoptotic effect.

2.1. *Intrinsic pathway of apoptosis*

The intrinsic pathway is also known as mitochondrial pathway in which pro-apoptotic stimulus affects molecular targets and finally results in an increase of the release of free cytosolic cytochrome *c*, which subsequently leads to apoptosome formation, caspase sequence activation, cleavage of targeting proteins and DNA fragmentation initiating and executing the process of apoptosis (*16*).

In the intrinsic pathway, Bcl-2 and Bax are of great importance. Apoptosis regulator Bax promotes apoptosis by binding to and antagonizing the Bcl-2 protein. The ratio of Bcl-2/Bax protein regulates the sensitivity of cells to apoptosis: the higher this ratio is, the less sensitive the cells are to apoptosis, and the opposite statement is also correct. When stimulated by the pro-apoptotic stimulus, the Bax protein could directly or indirectly interact with and induce the opening of the mitochondrial voltage-dependent anion channel resulting in the release of cytochrome c and other pro-apoptotic factors to promote apoptosis (*25*).

In the MCF-7 breast cancer cell line treated with 10-5 M resveratrol, the reduction of the Bcl-2/Bax ratio through enhancement of p53-dependent transcriptional activity at least partially contributes to resveratrol's pro-apoptotic activity in this research (*19*). A similar effect and mechanism could also be found in MCF-7 and MDA-MB231 breast cancer cells treated with resveratrol where the up-regulation of Bax and p21 resulted from the up- regulation of ASPP1 (apoptosis stimulation protein of p53 1) (*20*). In the prostate cancer cell line LNCaP, resveratrol (5, 10, and 25 μM) induced apoptosis more strongly than other wine polyphenols such as gallic acid, tannic acid and quercetin through significant enhancement of caspase 3 and 7 activity (*21*). It can be seen that the apoptosis of Caco-2 and HCT116 colon cancer cell lines was promoted after treatment with resveratrol (*23*), accompanied with a dose-dependent elevation of the expression of pro-apoptotic proteins cleaved caspase 7, cleaved caspase 9 and cleaved poly (ADP- ribose) polymerase (PARP). The activation of caspase 9 and 7 are crucial steps in apoptosis to induce the cleavage of PARP, a marker of cell apoptosis, to be an early DNA damage response. In other research also studied on these two cell lines, increased Bax/Bcl-2 ratio was found (*24*), suggesting the pro-apoptotic effect of resveratrol

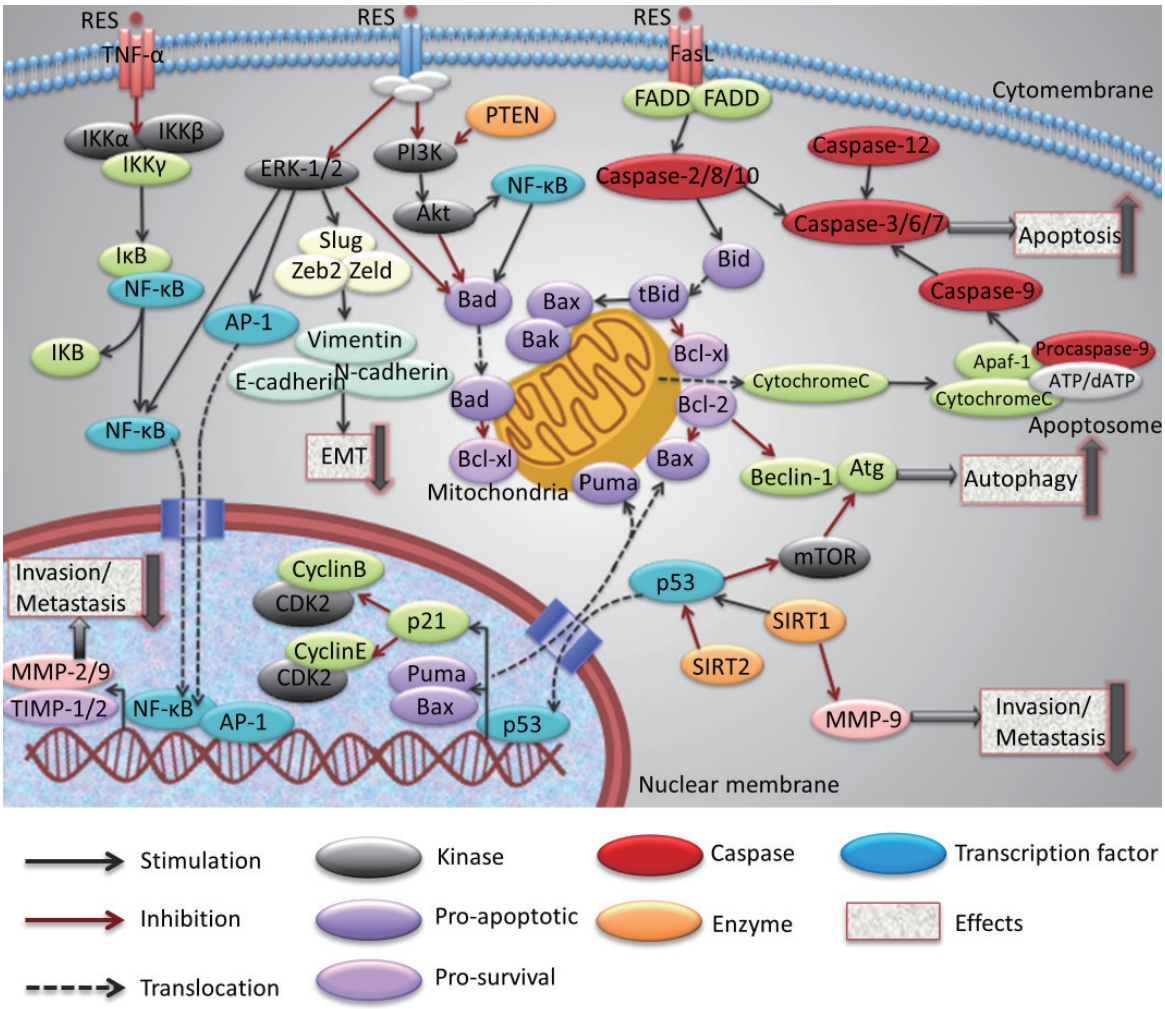

Figure 1. Anti-tumor effects and molecular mechanisms of resveratrol.

through influencing the intrinsic pathway. Treating ASTC-a-1 cells of human lung adenocarcinoma with 100 μM resveratrol (*25*) induced cell apoptosis specifically by intrinsic pathway as proved by the activation of caspase 3 and caspase 9 but not caspase 8 detected by fluorometric assay. A time-dependent loss of mitochondrial membrane potential was detected in combination with changes described above. Apoptosis-inducing factor (AIF) translocation into the nucleus was time-dependently elevated, and the knock down of AIF diminished resveratrol induced apoptosis, showing that in this research caspase 3 and 9 managed to induce apoptosis *via* the activation of AIF.

In vivo studies are consistent with the results derived from the *in vitro* studies. In an N-nitrosodiethylamine (DEN) induced hepatocellular carcinoma (HCC) of male Wistar rats (*26*), treatment with resveratrol either before or after the formation of HCC (20 mg/kg body weight for 15 days, from day 1 of DEN injection or the last two weeks after DEN injection) resulted in a decrease of α-fetoprotein and other known serum markers for HCC, such as aminotransferases, phosphatases, gamma-GT, and LDH. On the tissue level, H&E staining showed a remarkable difference in the tissue architecture compared to the untreated HCC model. Immunoblots showed that resveratrol prompted PARP cleavage, cytochrome *c* release, p53 expressioin and the conversion of procaspase 3 into active caspase 3 in HCC rat models. There was also elevation of Bax expression and drop of Bcl-2 expression at both transcriptional and translational levels. This research not only proved the equal effectiveness of the administration of resveratrol at the early or advanced stages of HCC, but also showed the relatively multilevel effects of this treatment. In another study of resveratrol's anti-cancer effect against HCC, the model was Sprague-Dawley rats with a single injection of diethylnitrosamine (DENA, 200 mg/kg) and subsequent promotion by phenobarbital (0.05%), the pre-administration of resveratrol (50, 100, 300 mg/kg body weight/day) dose-dependently reduced the incidence, total number and multiplicity of visible hepatocyte nodules (*27*). Mean nodule volume and its percentage of liver volume were also inhibited. Histopathological features were ameliorated and immunohistochemical detection revealed an increase of cell apoptosis. Bax expression was up-regulated while Bcl-2 was down-regulated, and

Table 1. Resveratrol against various types of cancer

Tumor types	Cell lines Animal models	Effects	Related mechanisms	*Ref.*
Breast cancer	MCF-7; MDA-MB231; BT474;	Apoptosis↑; Cell cycle arrest↑;	P53↑; P21↑; Bax/Bcl-2↑;	*19,20,38-40*
	4T1(BALB/C mouse model)	Cell proliferation↓; Cell invasion↓; Cell metastasis↓; EMT↓	Mitochondrial membrane potential↓; NF-κB↓; ERα↓; MMP-9↓	*57,66,67,71*
Hepatocellular carcinoma	HepG2; Hep3B; H4IIE;	Apoptosis↑; Cell cycle arrest↑;	Intrinsic/extrinsic apoptosis pathway↑; TIMP-1/2↑;	*18,26,27,*
	DENA-HCC model in SD rats	Cell proliferation↓; Cell invasion↓; Cell metastasis↓; Inflammation ↓	MAPK↓; NF-κB↓; FOXO3A↓; MMP-2/9↓; Hsp70↓; COX-2↓	*34-37,54,55, 59,64,80,81*
Colon cancer	HCT116; Caco-2; HT-29; DLD-1;	Apoptosis↑; Autophagy↑; Cell cycle arrest↑;	Intrinsic/extrinsic apoptosis pathway↑; P53↑;	*22-24,37,58,68, 79*
	SW480; COLO201; Lovo	Cell proliferation↓; Invasion↓; Metastasis↓; Glucose consumption↓	PI3K/AKT↓; ERK1/2↓; Wnt/β-catenin↓; MMP-7↓ NO↓; iNOS↓	
Prostate cancer	LNCaP; C42B; PC3; DU145	Apoptosis↑; Autophagy↑;	Intrinsic apoptosis pathway↑; PTEN↑; SIRT1↑;	*21,56,60,61,72*
		Cell proliferation↓; Invasion↓; Metastasis↓; EMT↓	p-AKT↓; E-cadherin↑ Androgen receptor (AR) ↓	
Lung adenocarcinoma/ NSCLC	ASTC-a-1; A549; H460	Apoptosis↑; Cell cycle arrest↑;	TRAIL receptor1/2↑; P53↑; P21↑; Intrinsic apoptosis pathway↑;	*25,41,42,69,73*
		Cell proliferation↓; Invasion↓; Metastasis↓; EMT↓	NF-κB↓; AP-1↓; MMP-9↓	
Gastric cancer	AGS; BGC-823; SGC-7901	Cell cycle arrest↑; Apoptosis↑; Senescence↑;	P21, P16↑; SIRT1↑;	*43,46*
		Cell proliferation↓; Invasion↓; Metastasis↓	Survivin↓; CyclinD1, CDK4,CDK6↓	
Esophageal cancer	EC109; EC9706; K562	Apoptosis↑; Autophagy↑;	Intrinsic apoptosis pathway↑; P53↑; Bax/Bcl-2↑	*47*
		Cell proliferation↓; Invasion↓; Metastasis↓		

they were demonstrated to be the mechanism of the anti-tumor effect of resveratrol in this HCC model.

As a whole, resveratrol could induce apoptosis in a variety of cancer types including HCC, breast cancer, lung cancer, colon cancer and prostate cancer through the intrinsic pathway of cell apoptosis, that is, some sort of stimulus disrupts the mitochondria membrane potential. This leads to the release of cytochrome *c* activating the caspases, which result in a cleavage of PARP and eventually, DNA fragmentation. Thus the whole process of the intrinsic apoptosis pathway finally causes cell apoptosis.

In order to improve the potency of resveratrol against tumor proliferation, several analogs of resveratrol have been synthesized. HS-1793, one of the analogs, showed stronger antitumor activity than resveratrol (*28*). It acted as a polyploidy inducer in prostate cancer LNCaP cells at a dose at which resveratrol couldn't induce multi-nucleation. During this process, caspase-3 degradation was detected, indicating that HS-1793 reduced the viability of LNCaP cells *via* the caspase-mediated pathway. An increase of p53 expression level was also detected in the polyploidy LNCaP cells. In a study about the higher hydroxylated resveratrol analogs (HHRA) on T cell leukemia Jurkat cells (*29*), the cytotoxic activity of 3, 30, 4, 40-tetrahydroxy-trans-stilbene (M6), 3, 4,

40, 5-tetrahydroxytrans-stilbene (M8) and 3, 30, 4, 40, 5, 50-hexahydroxy-trans-stilbene (M12) were compared with that of resveratrol, with IC_{50} values of 58.4 μM, 48.1 μM, 33.4 μM and 13.8 μM for Res, M6, M8, M12, respectively. Analogs possessing ortho-hydroxyl groups are stronger cytotoxic agents than compounds without this structure. The cytotoxicity was associated with the induction of oxidative stress in cancer cells. In research about the synthetic *cis*-polymethoxystilbenes (methylated analogs of *cis*-resveratrol) (*30*), they inhibited the proliferation and motility of melanoma cells with low micromolar specificity (IC_{50} < 10 μM) in contrast with the fact that both *trans*- and *cis*-resveratrol were ineffective at 10 μM. This effect was accompanied by a decrease of β-tubulin, a marker of metastatic melanoma cells. The increased anti-androgenic activity brought by a methoxy group on the C-4' of resveratrol and its analogs provided a more potent inhibition against prostate cancer cell LNCaP proliferation (*31*). In earlier studies about anti-androgen transcription, resveratrol was used at a concentration of 50 μM or higher, whereas in this study, the analogs were used at a concentration of 10 μM or less. Among them, 49-O-methylresveratrol (3, 5-dihydroxy-49-methoxystilbene) was the most effective one. Its stronger inhibition on Akt phosphorylation, which was related to androgen signaling was the underlying mechanism.

As for the structure-activity relationship, some studies were performed, demonstrating that the positions and numbers of hydroxy and methoxy groups were crucial for the inhibition effect of these components on SW480 and HepG2 tumor cells (*32*). The presence of a hydroxy group in a specific position and the presence of an increased inhibitory effect brought by a methoxy group were found in the analogs with an active tumor inhibition effect. In addition, at least one phenolic group was essential for the antitumor activity. These discoveries provided clues for further synthesis and research about resveratrol analogs against tumor cell proliferation.

2.2. *Extrinsic pathway of apoptosis*

The extrinsic pathway of apoptosis involves transmembrane receptor-mediated interactions, which are also called the TNF-induced (tumor necrosis factor) model and the Fas-Fas ligand-mediated model, both involving receptors of the TNF receptor (TNFR) family coupled to extrinsic signals (*33*). Upon ligand binding, cytoplasmic adapter proteins were recruited and associated with procaspase-8 *via* dimerization of the death effector domain, forming a death-inducing signaling complex (DISC) and resulting in the auto-catalytic activation of procaspase-8 (*34*). Downstream of the extrinsic pathway are the caspase sequence, separated as the initiator caspases, *e.g.* caspase 8, which was mentioned above, and the effector caspases, such as, caspase 3, 6, and 7. Effector caspases are activated by the initiator caspases activated to conduct the cell death program.

When H4IIE rat hepatoma cells are treated with 100 μM resveratrol for up to 24 h, activation of caspase 2 and 8/10 and consequently activation of caspase 3 were seen. No alteration of caspase 9 was detected. DNA fragmentation and formation of apoptotic nuclei were detected which demonstrated the induction of apoptosis by resveratrol in H4IIE cell line through the extrinsic pathway (*18*). In resveratrol treated colon cancer cell line HT-29, a concentration of 150 μM induced apoptosis in a time- dependent manner, and the activity of caspase 8/caspase 3 was increased without alteration of Bax or Bcl-2, which indicated that resveratrol induced apoptosis may be mediated through the death receptor pathway. The underlying mechanism is the enhancement of autophagy induced by resveratrol *via* increasing reactive oxygen species (ROS) production in HT-29, which was proved by the reduction of caspase 8/caspase 3 levels when the blocking of autophagy *via* 3-MA is conducted (*22*). In research on the colon cancer cell line of HCT116 and Caco2, resveratrol (100 μM) significantly activated the extrinsic apoptotic markers, caspase 3 and 8, with down-regulation of c-Myc and leptin (*24*).

In summary, the extrinsic pathway of resveratrol conducted on some cells express Fas or TNF receptors and can lead to apoptosis *via* ligand binding and protein cross-linking Finally, apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called "caspases" and a complex cascade of events that link the initiating stimuli to the final demise of the cell (*34*).

2.3. *Apoptosis led by cell cycle arrest: importance of p53 and p21*

Tumor protein p53, also known as p53, is crucial in multicellular organisms, where it regulates the cell cycle and, thus, functions as a tumor suppressor, preventing cancer. P53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation. It can activate DNA repair proteins when DNA has sustained damage, or initiate apoptosis – programmed cell death – if DNA damage proves to be irreparable. Activated p53 binds DNA and activates expression of several genes including microRNA miR-34a (*35*), WAF1/CIP1 encoding for p21, a member of cyclin-dependent kinase inhibitor playing a crucial role in cell growth arrest (*36*), and hundreds of other down-stream genes. P21 (WAF1) binds to the G1-S/CDK (CDK1, CDK2 and CDK4/6) complexes (responsible for the G1/S transition in the cell cycle) inhibiting their activity. Many studies demonstrated that the activation of p21 might be due to a p53-dependent pathway (*37*) and p21 has been shown to be essential for p53-mediated G1/S boundary cell-

cycle arrest and cellular senescence triggered by DNA damage (*38*).

In HCC cell line HepG2 treated with 10^{-7} M resveratrol, an up-regulation of endothelial nitric oxide synthase (eNOS) transcription leads to the subsequent activation of p53, causing a G1 and G2/M cell cycle arrest that finally promoted the HepG2 cell apoptosis (*39*). In this research, though not mentioned directly, we can assume that the G1 phase arrest of cell cycle was *via* the p21 activation induced by the activation and elevation of p53 as the mechanisms described previously. As for the cyclins that take control of cell cycle progression, p53 is not the only factor to affect them. For the HepG2 cells that were treated with 200 μM of resveratrol, a decrease in S phase associated with a concomitant increase in G1 phase of cell cycle distribution was found by flow cytometry (*40*). Apoptosis was elevated and the survival pathways, such as p38 MAPK, Akt and Pak1, were down-regulated both in their expression and activity. This was found to be the reason for the decrease of cyclin D1 either by up-regulating p53 or directly influencing cyclin D1 expression and eventually inducing cell cycle arrest and apoptosis, being the upstream regulators of p53 and cyclins. A similar result was also found in HepG2 cells treated with 10 or 20 μg/mL resveratrol for 24h accompanied with an elevation of p21 expression detected by ELISA (*41*), supporting our previous suppositon. In HepG2 and HCT116 (colon cancer) cells, resveratrol also caused p53 elevation and thus increased apoptosis (*42*).

Some of the up and down-stream regulators of this process have been revealed. In breast cancer cell line MCF-7 and MDA-MB231, the elevation of ASPP1, which was induced as the target gene of increased E2F-1, might contribute to the up-regulation of p53 target genes Bax and p21, thereby sensitizing breast cancer cells to resveratrol-induced apoptosis (*20*). In an experiment treating MDA-MB231 cells with resveratrol, the downstream p53-dependent pro-apoptotic genes including *p53*, *c-fos*, *c-jun*, *p21*, *PIG3*, and *BAD* induced by resveratrol were also confirmed (*43*). Upstream of this molecular pathway was also suggested to be resveratrol's binding to integrin αvβ3, which caused subsequent activation of the ERK and/or p38 kinase pathway to finally activate p53 (*45*). In gastric cancer cells like SGC7901, resveratrol induced survivin decrease could also lead to cell cycle arrest, such as G0/G1 phase up-regulation and S, G2/M phase distribution down-regulation (*51*).

As for the role of p53 as a transcription factor, resveratrol could reduce the Bcl-2/Bax ratio through regulating the Bcl-2 and Bax promoters by affecting transcription factors p53 and NF-κB differently: enhancing p53-dependent transcriptional activity and reducing the NF-κB-dependent transcriptional activity (*19*). In A549 non-small cell lung cancer (NSCLC) cells treated with benzopyrene and/or resveratrol, resveratrol down-regulated IKK and NF-κB, causing decrease in the expression of cyclinD1, in which process the up-regulation of p53 and p21 also contributed to it. This finally resulted in G2/M cell cycle arrest and increase of cell apoptosis (*46*).

Some experiments performed on tumor cells revealed two new approaches for p53 and p21 in inducing apoptosis: senescence and autophagy. Resveratrol induced premature senescence was found to be associated with increased expression of p53 and p21 in NSCLC cells (*47*), suggesting that the activation of the p53-p21 pathway may play an important role in resveratrol induced senescence. The senescence was also found to be related to the increase of NAPDH oxidase-5 (NOX-5)-mediated ROS up-regulation, from which we could suppose that it is the increase of ROS induced p53 and p21 overexpression and resulted in the latter effect: senescence. The inducement of senescence by p21 was detected in the gastric cancer cell AGS, too (*48*). Resveratrol inhibited cell viability and clonogenic potential, as well as arrested cell cycle in the G1 phase and led to senescence instead of apoptosis. The underlying mechanisms of this effect is the deregulation of the cell cycle and senescence pathway molecularly, including cyclin D1, CDK4 and 6, p21 and p16, since p21 and p16 signaling pathways could participate in senescence progression mediated by various kinds of stress as demonstrated by previous studies (*49,50*). Treatment with resveratrol on the esophageal squamous cell carcinoma cell line (*52*) resulted in increase of autophagic response, marked by significant elevation of LC3-II in autophagosomes, up-regulation of multiple key autophagosome-regulatory proteins, such as Beclin-1 and ATG5, and the formation of acidic vesicular organelles (AVO), which was consistent with the cell death effect of resveratrol. This was induced through p53 regulation: p53 target-damage-regulated autophagy modulator (DRAM) may regulate autophagy by affecting the fusion of autophagosomes and lysosomes as previously described. This suggested another way of p53 to control the cell apoptosis process.

There also exists some discussion about the dosage of resveratrol leading to cell cycle arrest-related apoptosis. In an experiment treating MDA-MB231 cells with resveratrol, it was found that 10 μM of resveratrol could reduce the percentage of proliferating cells to 33%, and even at a concentration as low as 0.1 μM, resveratrol could also induce more than a 50% decrease in cancer cell numbers compared to control group, which was in contrast to a previous study stating that the IC_{50} values for inhibiting cell growth by resveratrol would be in the range of 5 to 10 μM (*43*). In other research with IC_{50} of 60.5 μM to inhibit cell proliferation of MCF-7 cells (*44*), when treated with 30 μM of resveratrol, cell cycle showed a decrease of G0/G1 phase and G2/M phase

accompanied with an increase in the S phase. When elevating the dose up to 90 μM, there was a decrease of S phase and an increase in G0/G1 phase distribution compared with that of 30 μM, suggesting a biphasic effect of resveratrol on this cell.

Some resveratrol analogs were found to have a stronger growth inhibition effect than resveratrol by inducing cell cycle arrest. Phoyunbene (PYB) (*trans*-3, 4'-dihydroxy-2', 3', 5-trimethoxystilbene) strongly inhibited the growth of HepG2 cells with an IC_{50} of 37.1 μM compared with resveratrol (IC_{50}: 80.3μM) (*48*). The inhibition effect was due to PYB's induction of G2/M cell cycle arrest and apoptosis, which were associated with its up-regulation of cyclinB1 and Bax, as well as the down-regulation of Bcl-2. By the way, in a transwell experiment, it was found that PYB inhibits the invasion of HepG2 cells more strongly than resveratrol. These results support the notion that structure modification of resveratrol can increase its antitumor effects, though the underlying mechanism was not elucidated.

2.4. *Role of SIRT1 in apoptosis*

Mammalian ortholog of the yeast silent information regulator 2 (SIRT1) is a NAD-dependent histone deacetylase belonging to a multigene family of sirtuins that contains 7 members with distinct and diverse functions. SIRT1 can mediate cellular metabolism and energy production through regulation of forkhead box protein O1 (FOXO1) activity and insulin sensitivity and modulate inflammatory responses through NF-κB or cell growth through inhibition of mTOR activity. Besides, SIRT1 can also protect cells from apoptosis in response to genotoxic stress (*54-58*). In contrast, there are some data which indicate that SIRT1 possesses significant tumor suppressor activity. Increased SIRT1 could delay some kinds of tumor progression and protect the body or tissue from various diseases including cancer, cardiovascular abnormalities and metabolic syndrome-associated diseases as previous studies indicated (*59*).

In glucose (2.8, 5.5, and 25 mM)-exposed HepG2 cells, resveratrol treatment (100 μM) suppressed cell proliferation that could be induced by a high glucose concentration of 25 mM (*60*). On the molecular level, resveratrol induced the expression of SIRT1 that further involved the effects of resveratrol on the suppression of p-STAT3 and p-AKT as well as in the cell proliferation of HepG2 under high glucose conditions. The down-regulation of p-STAT3 and p-AKT caused decrease of cyclinD1, VEGF and MMP-9 in this process. In Hep3B cells stably expressing hepatitis B virus (HBV) treated with resveratrol (100 μM), ectopic expression and enhanced activity of SIRT1 were seen, which attenuated JNK phosphorylation, a prerequisite for resistance to oxidative stress-induced apoptosis (*61*). On the other hand, some research indicated that resveratrol might not activate but inhibit SIRT1 signal in HepG2 cells. The inhibition of SIRT1 increased p53 acetylation and enhanced expression of p53 downstream target p21 and activation of caspase-3, finally resulting in the increased S phase arrest of cell cycle and apoptosis. The inhibitory effect of resveratrol on sirtuin 1, which comes from the class III histone deacetylases (HDACs), raised a question of how resveratrol affects other HDAC classes. Research showed that resveratrol functioned as a pan-HDAC inhibitor in HepG2 cells and induced apoptosis.

In prostate cancer cell lines treated with resveratrol, SIRT1 expression enhanced more in androgen-independent prostate cancer cell lines (C42B, PC3, and DU145) than in androgen-responsive (LNCaP) or nontumorigenic prostate cells (RWPE-1), without any significant effect on SIRT1 enzymatic activity (*62*). Inhibition of SIRT1 using a shRNA promoted cell proliferation and inhibited autophagy by down-regulation of p-S6K and 4E-BP1. Resveratrol reversed these effects suggesting that targeting the SIRT1/S6K-mediated inhibition of autophagy represented an effective strategy of prostate cancer prevention. In the breast cancer cell line MCF-7, resveratrol, through P38 MAPK phosphorylation, caused induction of p53 that recruited at the estrogen receptor α proximal promoter to inhibit its expression both in mRNA level and protein level (*63*). The detailed mechanisms were as follows: a specific interaction of p53 and HDAC was found and the latter one was phosphorylated. The tripartite complex p53/Sin3A/HDAC1 together with NF-Y was phosphorylated and enhanced and was correlated with SP-1 and RNA polymerase II release, resulting in the inhibition of cell transcriptional activity including that of estrogen receptor α in breast cancer. In this process, HDAC1 phosphorylation could be critical for the formation of p53 and Sin3A-HDAC1 complexes at the promoter site that involve p53 binding. Also in colon cancer cell lines DLD-1, SW480 and COLO201 treated with resveratrol, SIRT1 was decreased *via* the elevated-miR-34a-induced decrease of its downstream target gene E2F3, accompanied by an inhibition of PI3K/Akt as the upstream modulator of miR-34a (*64*). These resulted in an induction of apoptosis in colon cancer cells. In prostate cancer cell line DU145 and PC3M, resveratrol inhibited the metastasis associated protein1 (MTA1)/HDAC unit that was indicated to be a negative regulator of PTEN (*67*). Thus acetylated PTEN was able to accumulate in the nucleus and rehabilitate resulting in diminished p-Akt levels, which facilitated inhibition of prostate cancer. The bilateral effect of SIRT1, as a tumor promoter or a tumor suppressor, need to be studied more to figure out the exact mechanisms or prerequisites to determine which effect SIRT1 performs, further supporting the anti-tumor potential application in the future.

2.5. *Other molecular events inducing apoptosis*

In the DENA-initiated hepatocarcinogenesis model of SD rats, the decease of myosin light chain kinase (MLCK) was found to be associated with the induction of apoptosis by resveratrol (*65*), saying that MLCK might be implicated in the formation of integrin-positive adhesive structures. However, further studies are needed to demonstrate how these kinases mediate integrin-dependent functions. In prostate cancer cells PC3, resveratrol-evoked cytosolic free Ca^{2+} concentration increases concentration-dependently by evoking phospholipase C-independent Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} entry *via* protein kinase C-regulated mechanisms (*66*). A low dose of resveratrol (1-10 μM) caused Ca^{2+} dependent cell proliferation, while at higher concentration it caused cell death, suggesting that in PC3 cells, resveratrol had a dual effect on cell viability.

3. Effects of resveratrol on tumor metastasis and invasion

3.1. *Resveratrol affects the expression and activity of MMPs*

The invasion and metastasis of cancer cells involve degradation of the environmental extracellular matrix (ECM) and basement membrane. This process is conducted through vatious proteolytic enzymes, such as Matrix metallopeptidase (MMPs). Among these enzymes, MMP-2 and MMP-9 are overexpressed in various malignant tumors to modulate cell invasion and metastasis (*68*). Both MMP-2 and MMP-9 enzymes are activated and capable of degrading type IV collagen, which is a major constituent of the basement membrane to ease cell mobility. On the other hand, tissue inhibitor metalloproteinase proteins (TIMPs) are a group of proteins consisting of TIMP-1, -2, -3 and -4 acting as natural MMP inhibitors (*69*). The effects of resveratrol on these molecular targets are summarized.

When treated with resveratrol (0-50 μM), the migration and invasion ability of phorbo-12-myristate 13-acetate (PMA)-treated HepG2 and Hep3B cell lines of HCC were both reduced in a dose-dependent manner. In HepG2 cells, the down-regulation of MMP-9 activity and up-regulation of TIMP-1 protein expression were found and in Hep3B cells, both the MMP-2 and MMP-9 activities were decreased accompanied with an increase in the protein expression level of TIMP-2 (*70*). These results suggested that resveratrol might exert anti-invasive and anti-migratory ability against hepatoma cells through regulation of MM-2, MMP-9, TIMP-1 and TIMP-2 activity and expression. In the process of regulating MMP-9, NF-κB was found to play an important role. The TNF-treated HepG2 cells expressed a high level of MMP-9, which was significantly suppressed by resveratrol through down-regulation of NF-κB expression resulting in downstream MMP-9 protein expression decrease and inhibition of invasion ability of HepG2 cells (*71*).

In breast cancer cell line MDA-MB231, resveratrol treatment (5 μM) inhibited the epidermal growth factor (EGF)-induced elevation of cell migration as well as expression of MMP-9. A subunit of the mammalian mediator complex for transcription called MED28, the overexpression of which could increase migration, was also reduced by resveratrol through the EGFR/PI3K signaling pathway (*72*). The *in vivo* research of breast cancer also confirmed the effect of inhibition of MMP-9 in the anti-metastasis process. BALB/c mice were injected with 4T1 cells with orally administered various concentrations of resveratrol (0, 100, 200 mg/kg body weight) (*73*). The numbers of pulmonary nodules were significantly decreased with a decreased plasma MMP-9 activity in response to the resveratrol treatment. A similar result was found in colorectal cancer cells LoVo and metastatic lung cancer cells A549 treated with resveratrol, resulting in invasion and metastasis inhibition (*74,75*). All together, resveratrol could inhibit invasion and metastasis both *in vitro* and *in vivo* through down-regulating the expression and activity of MMP-9.

3.2. *Resveratrol affects EMT*

Epithelial-to-mesenchymal transition (EMT) is thought to be a pivotal event in the initial step of the metastatic cascade allowing cells to acquire migratory, invasive and stem cell-like properties (*76*). Resveratrol has a significant effect on the process of EMT.

Resveratrol inhibits EGF induced EMT in breast cancer cell line MCF-7 through repressing EGF induced ERK activation (*77*), which resulted in the failure of EGF altering cell morphology, motility and EMT markers (Vimentin and N-cadherin) up-regulation. Transcription factors (Slug, Zeb1 and Zeb2) increased in EMT were also inhibited. Prostate cancer cells PC3 and LNCaP also experienced an EMT inhibition induced by resveratrol with a down-regulation of glioma-associated oncogene homolog 1 (Glil1), suggesting that the inhibition effect was realized through the Hedgehog signaling pathway (*78*). These provided a novel perspective on the role of resveratrol in preventing cancer progression as an EMT inhibitor. Experiments on the lung cancer cell line A549 also supported this conclusion (*79*).

Some studies suggested that we might not view the MMPs expression and the EMT process as separated parts. Over-expression of MMP-9 in A431-III cells might directly induce (or stimulate) EMT and the transcriptional factor, Snail, could cooperatively engage in this phenomenon as proved by using small interference RNA and MMP inhibitors (*70*). The up-regulation of MMP-2 and MMP-9 might initiate and

maintain long-term EMT (*80,81*). Considering their interactions and relationship, resveratrol's inhibition effect of them could be placed as a more important priority as an anti-cancer component.

4. Effects of resveratrol on inflammation

As previously mentioned, resveratrol has anti-oxidation, and anti-inflammation effects. An expending body of evidence suggests that inflammation-mediated processes, including the production of cytokines, chemokines, and reactive oxygen and nitrogen species may contribute to malignant cell transformation (*82,83*). A variety of studies have accumulated insights into the role of inflammation and oxidation in initiation, promotion and progression of cancers. In the process of tumor promotion activity by inflammation, components of the tumor microenvironment, such as tumor cells, stromal cells and infiltrated inflammatory/ immune cells generate an inflammatory state through proinflammatory expression and activation at an abnormal level. Many proinflammatory mediators, especially cytokines, and chemokines turn on the angiogenic switches mainly controlled by vascular endothelial growth factor, thus initiating inflammatory angiogenesis. This ends up with tumor angiogenesis, metastasis and invasion, upregulating the malignancy level of tumors (*82*).

Resveratrol can inhibit the inflammation response of colon cancer cell lines Caco-2 and SW480 induced by lipopolysaccharide (LPS) *via* inhibition of the NF-κB signaling pathway. Though the detailed mechanisms still need to be studied, it can be supposed that resveratrol inhibits NF-κB through a direct action on the nuclear transcription factor *via* phosphorylation inhibition, or by TLR-4 down-regulation (*84*).

The anti-inflammation effect of resveratrol was supported by research *in vivo* as well. In the DENA induced HCC model in SD rats, a 20-week administration of resveratrol (50, 100, 300 mg/kg) was shown to inhibit hepatocyte nodules in a dose-dependent manner (*85*) through the down-regulation of HSP70 and COX-2 expression by attenuating the translocation of NF-κB from the cytoplasm to the nucleus. In another study with the same administered dose of resveratrol, it was also found that the level and expression of hepatic TNF-α, IL-1β and IL-6 induced by DENA was reversed (*86,87*). Thus resveratrol mediated an anti-tumor effect of rat liver tumor *via* alteration of proinflammatory cytokines.

There was also research concerning resveratrol analogs that showed more activity in inhibiting inflammation. Polyhydroxy and polymethoxy substituted analogs of resveratrol were synthesized and some of them exhibited more potent anti-tumor and anti-inflammatory effects, such as hexahydroxystilbene (M8)'s down-regulating COX-2 and inducing apoptosis with a much lower concentration than resveratrol (*88*). A structure-activity study showed that increasing the number of OH groups and their position on the phenol ring could increase the ability of free radical scavenging, and the orthosemiquinones formed during metabolism or autoxidation enabled the analogs to have a stronger cytotoxic effect.

5. Conclusions

The current evidence for anti-tumor effects of resveratrol in certain types of cancers, including lung, liver, stomach, colorectal, breast and esophageal cancer, assessed with *in vitro* and *in vivo* research is reported. Resveratrol exhibits the anti-tumor effect through modulation of the apoptotic pathways, such as intrinsic pathway, extrinsic pathway, cell cycle arrest pathway and the pathways affecting SIRT, and also the pathways regulating cell invasion and metastasis abilities. In addition, the resveratrol anti-inflammatory effect is of great significance in the inhibition of tumor initiation and progression. The results obtained in the present research, in which resveratrol was evaluated as an anti-cancer natural component, are encouraging. However, further study is needed to define the effectiveness of resveratrol in the prevention and treatment of these cancers. Pharmacokinetic studies of resveratrol have revealed its poor bioavailability, which urge research on resveratrol analogs that could improve the beneficial effects. Research showed that proper structural change of chemical groups, such as the OH group, might increase the antitumor effect. These analogs have shown enhanced ability of anti-proliferation, anti-invasion and anti-inflammation. The anti-tumor effects of resveratrol analogs and the studies about the structure-activity relationship of them have brightened the future of resveratrol's application in cancer therapeutics and their transfer into clinical applications. Even if there are still unknown facts and unsolved problems to be faced, we can conclude that resveratrol holds promising anti-tumor potential against various cancers and it deserves further research and evaluation in the future.

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