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The anticancer effects of resveratrol – Modulation of transcription factors

Nichelle C. Whitlock and **Seung Joon Baek***
Laboratory of Environmental Carcinogenesis, Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

Abstract

Resveratrol (3, 4', 5-trihydroxystilbene), a naturally-occurring phytoalexin readily available in the diet, is reported to possess both chemopreventive and chemotherapeutic activities in several cancers. However, despite the identification of numerous molecular targets, the underlying mechanisms involved in the anticancer activities of resveratrol are not completely understood. Resveratrol is postulated to function as a potential signaling pathway modulator and as such, is demonstrated to affect a multitude of signal transduction pathways associated with tumorigenesis and/or carcinogenesis; it is likely that this collective activity, rather than just a single effect, may play an important role in the anticancer properties of resveratrol. Since transcription factors control the expression of many genes, the elucidation of molecular targets of resveratrol involved in transcriptional regulation is necessary to better understand how this dietary phytochemical affects chemopreventive and chemotherapeutic processes. As a result, investigators have increasingly searched for and examined possible targets of resveratrol. In this review, we summarize the current knowledge on molecular targets, specifically transcription factors, that contribute to the observed anticancer effects of resveratrol related to: (1) inhibition of carcinogenic activation and induction of carcinogen detoxification, (2) induction of growth arrest and apoptosis, and (3) suppression of pro-inflammatory signaling pathways related to cancer progression.

Keywords

AhR; ATF3; Experimental; FOXO; Resveratrol

INTRODUCTION

Epidemiological and current laboratory studies suggest consumption of certain types of fruits and vegetables, containing phytochemicals, is associated with reduced cancer risk (1). Furthermore, it is postulated that dietary phytochemicals can function as chemopreventive and/or adjuvant chemotherapeutic agents. One such phytochemical is resveratrol (3, 4', 5-trihydroxystilbene), a naturally-occurring phytoalexin readily available in the diet and to which a plethora of health-promoting effects have been ascribed. Resveratrol, first identified as a bioactive compound in 1992, is found in several plants, particularly in the skin of red grapes (2). This compound has elicited much attention as a potential anticancer agent since its inhibitory effect on carcinogenic processes (initiation, promotion, and progression) was first reported in 1997 (3). Subsequently, numerous studies, using both *in vitro* and *in vivo* model systems, have illustrated resveratrol's capacity to modulate a multitude of signaling

* *Corresponding Author:* Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Dr., Knoxville, TN 37996. Tel.: +1 865 974 8216; fax: +1 865 974 5616. sbaek2@utk.edu (S.J. Baek)..

pathways associated with cellular growth and division, apoptosis, angiogenesis, invasion, and metastasis (4).

Despite substantial progress in understanding the molecular basis of resveratrol's anticancer activities, few clinical trials have been undertaken to confirm its use as a chemopreventive and/or adjuvant chemotherapeutic agent. Preclinical studies have demonstrated the inhibitory effects of resveratrol in different cancers (5) and its ability to act as an adjuvant to traditional chemotherapeutics (6-9). In this review, we summarize the current knowledge on molecular targets of resveratrol, specifically transcription factors, that contribute to observed anticancer effects of this dietary phytochemical; these transcription factors include the aryl hydrocarbon receptor (AhR), nuclear factor E2-related factor 2 (Nrf2), p53, forkhead box subgroup O (FoxO), nuclear factor-κB (NF-κB), and activating transcription factor 3 (ATF3).

ARYL HYDROCARBON RECEPTOR AND NUCLEAR FACTOR E2-RELATED FACTOR 2

Oxidative metabolism by phase I enzymes, such as those belonging to the cytochrome P450 (CYP) family, results in the conversion of pro-carcinogens to reactive electrophilic intermediates, which are further metabolized by phase II enzymes via the conjugation of hydrophilic moieties. Resultant metabolites are detoxified and eliminated. However, inadequate detoxification by phase II enzymes potentiates genotoxicity of phase I products, thus initiating the carcinogenic process (10). Reports of modification of both phase I and phase II xenobiotic metabolizing enzymes by resveratrol suggest an explanation for the compound's chemopreventive effect (Fig. 1). In this regard, two primary molecular mechanisms have been identified: (1) inhibition of AhR-mediated activation of phase I enzymes and (2) induction of Nrf2-mediated activation of phase II enzymes.

Aryl hydrocarbon receptor (AhR)

Resveratrol is reported to alter phase I enzyme expression and activity in both an AhR-dependent and AhR-independent manner (11). In this review, we focus on resveratrol-mediated repression of AhR-induced phase I enzyme expression. The canonical AhR-dependent signaling pathway is thought to contribute to carcinogenic initiation by phase I enzyme-activated polycyclic aromatic hydrocarbons (PAH), and inhibition of AhR signaling by resveratrol is thought to suppress this initiative process. Under basal conditions, unbound AhR forms a multimeric complex in the cytosol. However, upon ligand binding (e.g. binding by PAHs), AhR translocates to the nucleus (shedding the multimeric complex), forms a heterodimer with the AhR nuclear translocator (ARNT), and binds to gene promoters containing xenobiotic response elements (XRE) resulting in the trans-activation of phase I enzymes (12).

Several reports demonstrate the inhibitory effects of resveratrol on AhR-mediated activation of phase I enzymes. For example, resveratrol was shown to impair TCDD (2, 3, 7, 8-tetrachlorodibenzo-*p*dioxin)-induced recruitment of AhR and ARNT to the *CYP1A1/1B1* and *CYP1A1/1A2* promoter in MCF-7 breast and HepG2 liver cancer cells, respectively, resulting in decreased expression (13). Resveratrol also effectively blocked TCDD-induced, AhR-dependent transcription in both an estrogen receptor (ER)-dependent and ER-independent manner (14, 15). Using ER-positive cancer cells, Perdew *et al.*(14) observed that at micro-molar concentrations, resveratrol reduced the AhR/ARNT complex at the *CYP1A1* promoter, resulting in near-complete inhibition of CYP1A1 expression and metabolic activity; however, at nano-molar concentrations, resveratrol repressed AhR-mediated induction of CYP1A1 without interfering with AhR association with XREs after

TCDD exposure. On the other hand, MacPherson and Matthews (15) showed resveratrol's competitive displacement of TCDD from AhR, indicating prevention of TCDD-induced CYP1A1/1B1 expression mediated by AhR/ARNT complex recruitment to the promoter in both ER-positive and ER-negative breast cancer cells. Furthermore, resveratrol was demonstrated to reverse TCDD-induced, AhR-mediated CYP1A1 and matrix metalloproteinase 9 expression in the gastric cancer cell line AGS (16). Thus, resveratrol plays a suppressive role in AhR-mediated activation of phase I enzymes, contributing to the anticancer activity of this dietary phytoalexin.

Nuclear factor E2-related factor 2 (Nrf2)

Induction of Nrf2 signaling by resveratrol is thought to confer protection against phase I enzyme-activated carcinogens and associated carcinogenicity via the trans-activation of antioxidant and phase II detoxifying enzymes. Under basal conditions, Kelch-like ECH-associated protein 1 (Keap1) sequesters Nrf2 in the cytoplasm, targeting the transcription factor for proteasomal degradation. However, when induced by electrophiles, reactive oxygen species, or dietary phytochemicals such as resveratrol, Nrf2 dissociates from Keap1 and translocates to the nucleus where it dimerizes with small Maf proteins and activates antioxidant response element (ARE)-driven gene promoters (17).

Bishayee *et al.* (18) demonstrated that attenuation of DENA (diethyl nitrosamine)-induced liver carcinogenesis by resveratrol was mediated by increased Nrf2 expression. Resveratrol induction of NAD(P)H quinone oxidoreductase (NQO1) expression in TCDD-treated MCF10F immortalized breast cells involved Nrf2, leading to the suppression of DNA adduct formation (19). NQO1 was also increased by resveratrol after 4-hydroxyestradiol and estradiol-3, 4-quinone treatment (20). Induction of Nrf2 signaling by resveratrol resulted in increased expression of NQO1, heme-oxygenase 1 (HO-1), and glutamate cysteine ligase catalytic subunit in cigarette smoke extract-treated bronchial epithelial cells (21). Kode *et al.* (22) observed restored glutathione levels in cigarette smoke extract-treated A549 lung alveolar epithelial cancer cells by resveratrol; this effect was mediated via Nrf2-induced glutamate cysteine ligase expression and activity through the inhibition of cigarette smoke extract-modified Nrf2 post-translation. Resveratrol protected primary hepatocytes exposed to oxidative stress via increased Nrf2-mediated NQO1, catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase expression (23). Furthermore, resveratrol increased NQO1 expression and activity in the K562 leukemia cell line, which was associated with resveratrol-induced Nrf2/Keap1 complex disruption, Nrf2 nuclear translocation, and subsequent binding to ARE within the *NQO1* promoter (24). These results indicate that Nrf2 is a key protein that controls resveratrol-induced anti-tumorigenesis in several cancers. In contrast, Kawai *et al.* (25) observed Nrf2 cytoplasmic accumulation and inhibition on Nrf2-dependent transcription by resveratrol, presumably mediated through induced SIRT1 deacetylase activity, in both K562 leukemia and HepG2 hepatocellular carcinoma cell lines. Thus, resveratrol could affect not only translocation but also accumulation of Nrf2.

P53 TUMOR SUPPRESSOR

The tumor suppressor protein p53 is a critical transcription factor involved in the regulation of cell proliferation and apoptosis; as such, it is a key mediator in the prevention of carcinogenesis (26). It has been shown that resveratrol can cause apoptosis through p53-dependent and p53-independent pathways (27). Herein, we highlight reported resveratrol-induced, p53-mediated anticancer mechanisms.

Several groups, including our laboratory, have implicated the activation of p53-dependent pathway(s) in the observed anti-proliferative effects of resveratrol. For example, Heiss *et al.*

(28) demonstrated that chronic administration of resveratrol at a sub-apoptotic dose resulted in senescent-like growth arrest in different carcinoma cell lines. This effect was due to increased reactive oxygen species generation, ataxia telangiectasia mutated kinase (ATM) and p53 activation (via p38MAPK-mediated p53 phosphorylation at serine 15), induction of p21, and subsequent induction of senescence. Resveratrol is also reported to induce apoptosis via activation of both intrinsic (mitochondria-mediated) and extrinsic (death receptor-mediated) pathways (27). Resveratrol altered the Bax:Bcl2 ratio in the A431 epidermoid cancer cell line, leading to mitochondrial membrane depolarization and subsequent induction of caspase-dependent apoptosis, presumably mediated through p53 activation (29). Using MCF-7 breast carcinoma cells, Singh *et al.* (30) reported resveratrol-induced G0/G1 and S phase growth arrest associated with p53 phosphorylation at serine 15, increased expression of p53-regulated pro-apoptotic proteins (p21, Bax, and Fas), caspase 8/9 activation, and decreased Bcl2 expression; similar results were observed with resveratrol and cyclophosphamide co-treatment. Furthermore, Bishayee and Dhir (31) demonstrated reduced tumor incidence resulting from resveratrol-induced apoptosis associated with increased Bax:Bcl2 ratio (increased Bax and decreased Bcl2 expression) in a diethylnitrosamine-initiated, phenobarbital-promoted *in vivo* model of liver carcinogenesis. Resveratrol also increased p53-mediated expression of pro-apoptotic proteins (e.g. Bax, Bak, Bim, PUMA, Noxa, etc.) and the release of mitochondria proteins (e.g. cytochrome *c*, Smac/DIABLO, etc.) to the cytosol, thus triggering suppression of inhibitors of apoptosis proteins (e.g. Bcl2, Bcl-XL, survivin, XIAP, etc.) and caspase activation in several cancers (9, 32-34).

How resveratrol increases p53 expression is not clear; it is believed that resveratrol treatment causes DNA damage, which facilitates p53 activation. Tyagi *et al.* (35) observed resveratrol-induced cell cycle arrest was mediated through increased Cdc2 tyrosine 15 phosphorylation via the activation of the DNA damage pathway (i.e. ATM/ATR-Chk1/2-Cdc25 pathway). Resveratrol treatment also suppressed the metastasis-associated protein-1/nucleosome remodeling deacetylation complex, allowing for increased p53 acetylation and subsequent activation of pro-apoptotic genes (36). Moreover, our laboratory reported that resveratrol-induced expression of the pro-apoptotic protein non-steroidal anti-inflammatory drug-activated gene-1 is mediated by p53, resulting in induction of cell death (37).

FORKHEAD BOX, SUBGROUP O (FOXO) TRANSCRIPTION FACTORS

Proteins known as FoxO, divergent members of the Fox/winged-helix transcription factor superfamily, are recognized tumor suppressors that play a critical role in cell fate decisions (38). Composed of four members (FoxO1, 3, 4, and 6), FoxO transcription factors coordinate the expression of genes involved in diverse cellular processes, including cell cycle progression, apoptosis, and oxidative stress response. The function of FoxO proteins is regulated by post-translational modification(s), specifically phosphorylation and acetylation, which dictates their subcellular localization and transcriptional activity (39).

Abrogation of FoxO function occurs in numerous cancers due in large part to the constitutive activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, a key regulator of FoxO activity. PI3K/Akt-mediated phosphorylation results in the inactivation of FoxO transcription factors. FoxO phosphorylation by PI3K/Akt facilitates their interaction with 14-3-3 chaperone proteins and nuclear export; cytoplasmic sequestration inhibits FoxO-dependent transcription (38). FoxOs are also regulated by de/acetylation in response to oxidative stress. Several studies identify FoxOs as targets of the NAD-dependent class III histone/protein deacetylase sirtuin 1 (SIRT1). Under conditions of oxidative stress, SIRT1 forms a complex with and deacetylates FoxO transcription factors, resulting in the preferential activation of cell cycle arrest/stress resistance-related genes, thereby promoting

cell survival (40-42). Furthermore, silencing of SIRT1 resulted in FoxO4-mediated apoptosis in epithelial-derived cancer cells (43). Thus, targeting these inhibitory pathways may prove advantageous in the prevention/treatment of cancers.

The activation of FoxO transcription factors is implicated in the observed anticancer activities of resveratrol. Using prostate cancer cells, Chen *et al.* (44) demonstrated resveratrol's ability to inhibit the phosphorylation of PI3K/Akt (i.e. PI3K/Akt inactivation) resulting in decreased FoxO phosphorylation. Resveratrol increased nuclear translocation, DNA binding affinity, and transcriptional activity of FoxOs. Furthermore, the anti-proliferative effect (Bim/TRAIL/DR4/DR5/p27KIP1 induction and cyclin D1 inhibition) of resveratrol on prostate cancer cells is FoxO-dependent. Similar results were also observed *in vivo* (45). Additionally, the anti-migratory/angiogenic effects of resveratrol observed in human umbilical vein epithelial cells were FoxO-dependent and predicated on PI3K/Akt pathway inhibition (46). A schematic representation of resveratrol-mediated FoxO regulation is shown in Fig. 2.

As described, SIRT1-induced FoxO deacetylation results in the preferential activation of cell survival-related genes. Being that resveratrol is considered a SIRT1 agonist, although currently controversial, one could speculate the activation of a FoxO-mediated pro-survival mechanism induced by resveratrol may occur through increased SIRT1 deacetylase activity. Indeed, this is demonstrated in cardiomyocytes; resveratrol, presumably through SIRT1, increased FoxO transcriptional activation of stress resistance-related genes and subsequent phosphorylation by Akt (47). However, to the best of our knowledge, the resveratrol/SIRT1/FoxO signaling axis has not been studied in cancer cells thus allowing one to infer such an effect is context dependent.

NUCLEAR FACTOR-κB (NF-κB)

The link between inflammation and cancer is well established; these inflammatory processes contribute to the development and progression of carcinogenesis, including tumor growth, angiogenesis, invasion, and metastasis (48). A key mediator of inflammation-induced cellular transformation is the transcription factor NF-κB. Cytoplasmic sequestration by IκBα prevents NF-κB-dependent trans-activation. Upon activation, IKKβ phosphorylates IκBα, resulting in its degradation, and facilitates NF-κB nuclear translocation and subsequent activation of transcription (49).

It has been postulated that the anticancer effects of resveratrol are attributable to the inactivation of NF-κB-dependent signaling. Resveratrol inhibited IKKβ-mediated IκBα phosphorylation, resulting in increased IκBα expression, NF-κB cytoplasmic retention, and subsequent NF-κB inactivation (50, 51). Resveratrol also blocked interleukin-1β (IL-1β)-, tumor necrosis factor α (TNF-α)-, and HO-1-induced NF-κB activation (52-54). Resveratrol treatment of multiple myeloma cells resulted in cell cycle arrest and suppression of NF-κB-dependent signaling related to proliferation (cyclin D1), survival (Bcl2, Bcl-XL, XIAP, c-IAP, and survivin), angiogenesis (vascular endothelial growth factor), and metastasis (matrix metalloproteinase 9 and IL-6) (6, 55). Similar results were observed *in vivo* using a DENA-initiated liver carcinogenesis model (56) and Mia PaCa-2 orthotopic model of pancreatic cancer (8). Furthermore, resveratrol prevented PMA (phorbol 12-myristate 12-acetate)-induced HT1080 fibrosarcoma cell adhesion to endothelial cells via modulation of intercellular adhesion molecule-1 expression and NF-κB activity (57). Taken together, it is clear that resveratrol not only curbs expression of NF-κB, but also impedes the phosphorylation of IκBα thereby keeping the constitutive NF-κB subunit in an inactive state, resulting in suppression of the inflammatory and pro-tumorigenic changes associated with this pathway (Fig. 3).

ACTIVATING TRANSCRIPTION FACTOR 3 (ATF3)

Recently, our laboratory identified ATF3 as a novel molecular target of resveratrol in colorectal carcinoma cells (58). ATF3, a member of the ATF/CREB family of transcription factors, is characterized as a stress-inducible or adaptive response gene (59). Much controversy exists as to the role of ATF3 in tumorigenesis, and ATF3 is demonstrated to be a positive or negative modulator of tumor progression. However, several lines of evidence suggest that ATF3 may function as a tumor suppressor gene in colorectal carcinogenesis. Firstly, ATF3 expression is markedly reduced in cancer tissues when compared to normal adjacent tissue (60). Secondly, ATF3 over-expression is demonstrated to elicit a number of cellular responses, including inhibition of proliferation (61), induction of apoptosis (62-65), inhibition of invasion and associated genes (66-68), and retardation of tumor formation *in vivo* (63, 66). Finally, ATF3 is reported to mediate or enhance induction of apoptosis by compounds demonstrated to possess anti-tumor properties (64, 69-72). Thus, it is believed that ATF3 plays an anti-tumorigenic role in colorectal cancer.

As stated, we identified ATF3 as a novel target of resveratrol in colorectal cancer cells and showed resveratrol involvement in the transcription factor's regulation. Specifically, resveratrol-induced ATF3 expression is mediated by the induction and interaction of C2H2-type zinc finger transcription factors early growth response-1 (Egr-1) and Krüppel-like factor 4 (KLF4). Egr-1 and KLF4 belong to a family of immediate early response transcription factor genes whose expression is transiently induced in response to various environmental stimuli (73, 74). Both Egr-1 and KLF4 are suggested to act as master regulatory proteins involved in cell fate decisions (75, 76); as such, these transcription factors coordinate the expression of genes associated with cell proliferation, differentiation, and apoptosis (74, 77, 78). Nonetheless, as with ATF3, much controversy exists as to the role of Egr-1 and KLF4 in cancer development, and their biological function appears largely context dependent. Several studies have demonstrated that Egr-1 and KLF4 expression facilitates tumor progression *in vivo* (79-81); however, there is ample evidence supporting a tumor suppressive role for both transcription factors (82-88). Because resveratrol can increase Egr-1 and KLF4 expression and transcription activity, one could speculate that observed anticancer properties of resveratrol may be facilitated through either an Egr-1-mediated or KLF4-mediated mechanism (Fig. 4). Furthermore, increased ATF3 expression by resveratrol facilitated induction of apoptosis, at least partially, by the dietary compound (58). However, continued validation of ATF3 as a target of resveratrol is necessary to determine if such phenomena are specific to colorectal cancer cells or can be observed in other cancer phenotypes, both *in vitro* and *in vivo*.

CONCLUDING REMARKS

The continued identification of dietary phytochemicals and their related derivatives with chemopreventive and/or chemotherapeutic activities offers an alternative and complementary approach to the prevention and treatment of cancers. Studies investigating the use of these compounds for cancer prevention or as adjuvants to traditional treatment have revealed several potential benefits: (1) suppression of tumorigenesis and carcinogenesis *in vitro* and *in vivo*, (2) sensitization of cancer cells to drug-induced growth inhibition, and (3) minimization of adverse effects associated with conventional therapies. Most importantly, dietary phytochemicals are demonstrated to target multiple signal transduction pathways involved in tumorigenesis and carcinogenesis, an important advantage due to the inherent heterogeneity of cancers. Thus, it is not surprising that the number of studies involving dietary phytochemicals, such as resveratrol, has dramatically increased in the past decade.

As discussed in previous sections, resveratrol is demonstrated to modulate the expression and/or activity of transcription factors involved in critical pathways of carcinogenesis, including carcinogen activation and detoxification, growth arrest and apoptosis, and pro-inflammatory-mediated signaling pathways (e.g. inflammation-promoted cell proliferation, angiogenesis, invasion, and metastasis). Resveratrol is demonstrated to participate in both pro-survival and pro-death cellular mechanisms, either by favoring the preservation of the functional status of cells and possibly elongating cellular life span or inducing death of those cells whose physiological conditions have become deranged. Resveratrol also affects the transcriptional machinery resulting in trans-activation of key regulatory proteins. Thus, resveratrol may function as a signaling pathway modulator and as such, is demonstrated to affect a multitude of signal transduction pathways associated with tumorigenesis and/or carcinogenesis through the alteration of key transcription factors. Yet, it is likely that the observed anticancer properties of resveratrol are due to the collective modification of numerous signaling pathways, rather than just a single effect. Because transcription factors control the expression of many genes, the elucidation of molecular targets of resveratrol involved in transcriptional regulation is necessary to better understand how this dietary phytochemical affects chemopreventive and chemotherapeutic processes. As a result, researchers continue to investigate the molecular and cellular effects of resveratrol in cancer in hopes of unraveling the mysteries of this fascinating and promising dietary molecule.

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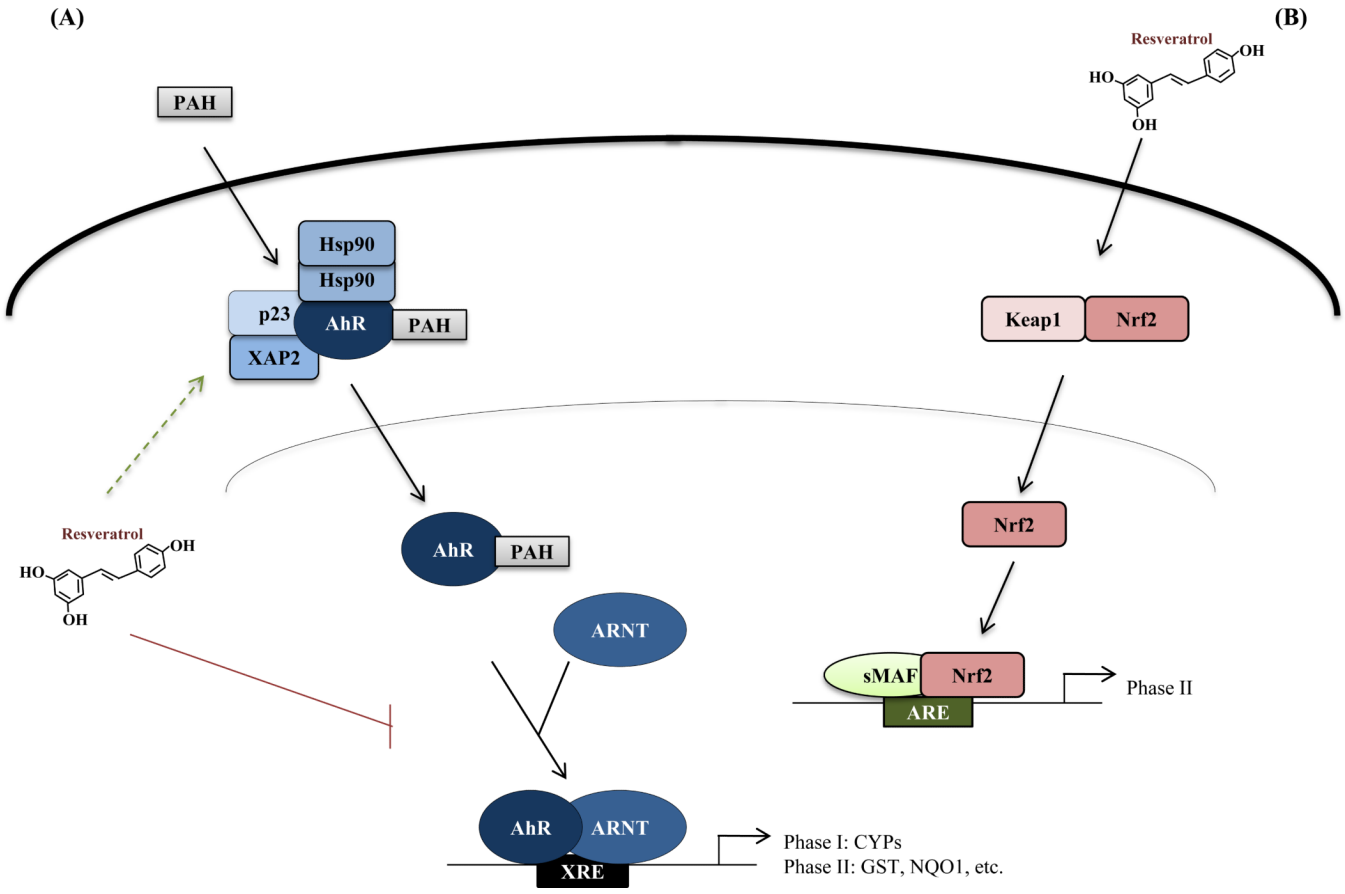

Fig. 1. Effects of resveratrol on AhR/Nrf2 signaling pathways

For a detailed description of AhR and Nrf2 signaling, refer to the text. Briefly, (A) PAH binds AhR (bound by the complex) and facilitates AhR translocation to the nucleus, where it forms a heterodimer with ARNT. The AhR/ARNT heterodimer then binds and trans-activates XRE-driven phase I/II enzyme promoters and initiates carcinogenesis (12). Resveratrol is demonstrated to inhibit AhR/ARNT recruitment to the promoter (red line; see text for references) and is speculated to displace PAH ligand binding and stabilize the cytosolic AhR complex (dotted green arrow; see text for reference). (B) Resveratrol-induced Nrf2 signaling confers protection against activated phase I enzymes (see text for references). Resveratrol promotes Nrf2 dissociation from Keap1 and nuclear translocation. In the nucleus, Nrf2 forms a heterodimer with small Maf proteins and trans-activates ARE-driven gene promoters (17).

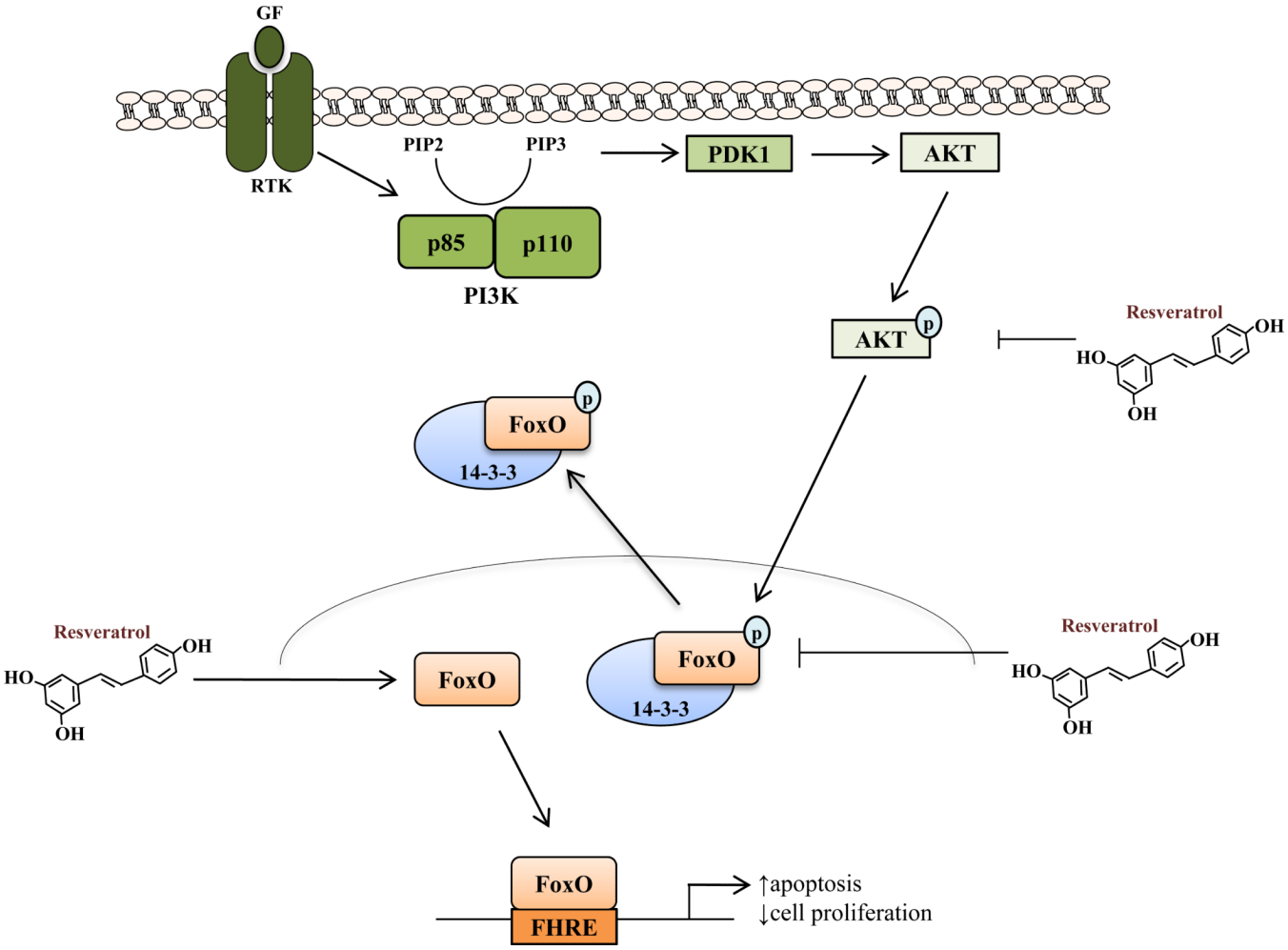

Fig. 2. Effects of resveratrol on the FoxO signaling pathway

PI3K/Akt-mediated phosphorylation results in the inactivation of FoxO transcription factors. FoxO phosphorylation by PI3K/Akt facilitates FoxO interaction with 14-3-3 chaperone proteins and nuclear export; cytoplasmic sequestration inhibits FoxO-dependent transcription (38). Resveratrol blocks Akt activation and subsequent FoxO phosphorylation and nuclear export; on the other hand, resveratrol-induced FoxO expression facilitates trans-activation of anti-proliferative/pro-apoptotic forkhead response element (FHRE)-driven promoters (see text for references). GF, growth factor; RTK, receptor tyrosine kinase.

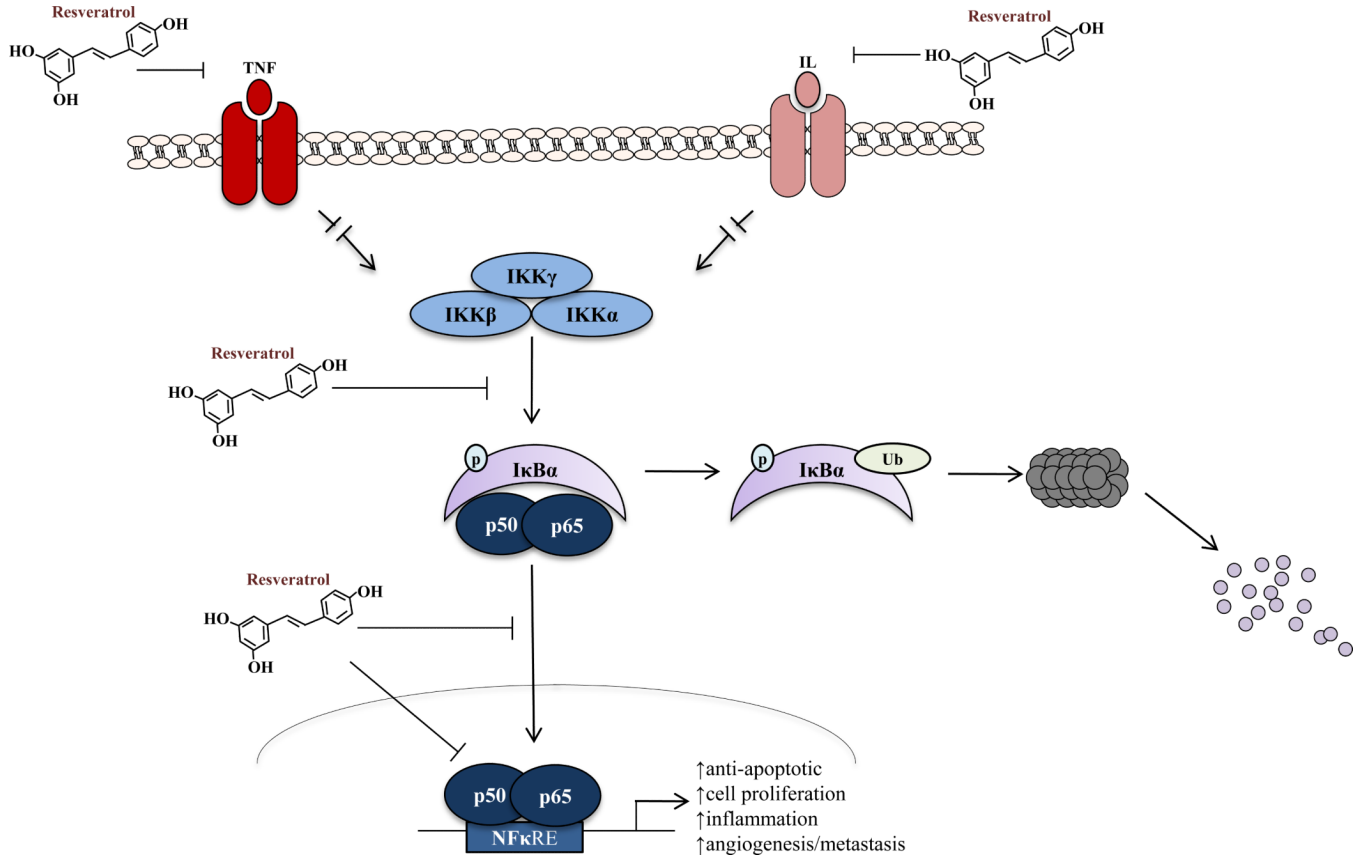

Fig. 3. Effects of resveratrol on the NF-κB signaling pathway

Activation by cytokine results in IKK phosphorylation of IκBα, resulting in IκBα degradation, and facilitation of NF-κB nuclear translocation and subsequent activation of transcription (49). Resveratrol is demonstrated it inhibit NF-κB signaling at all steps (see text for references).

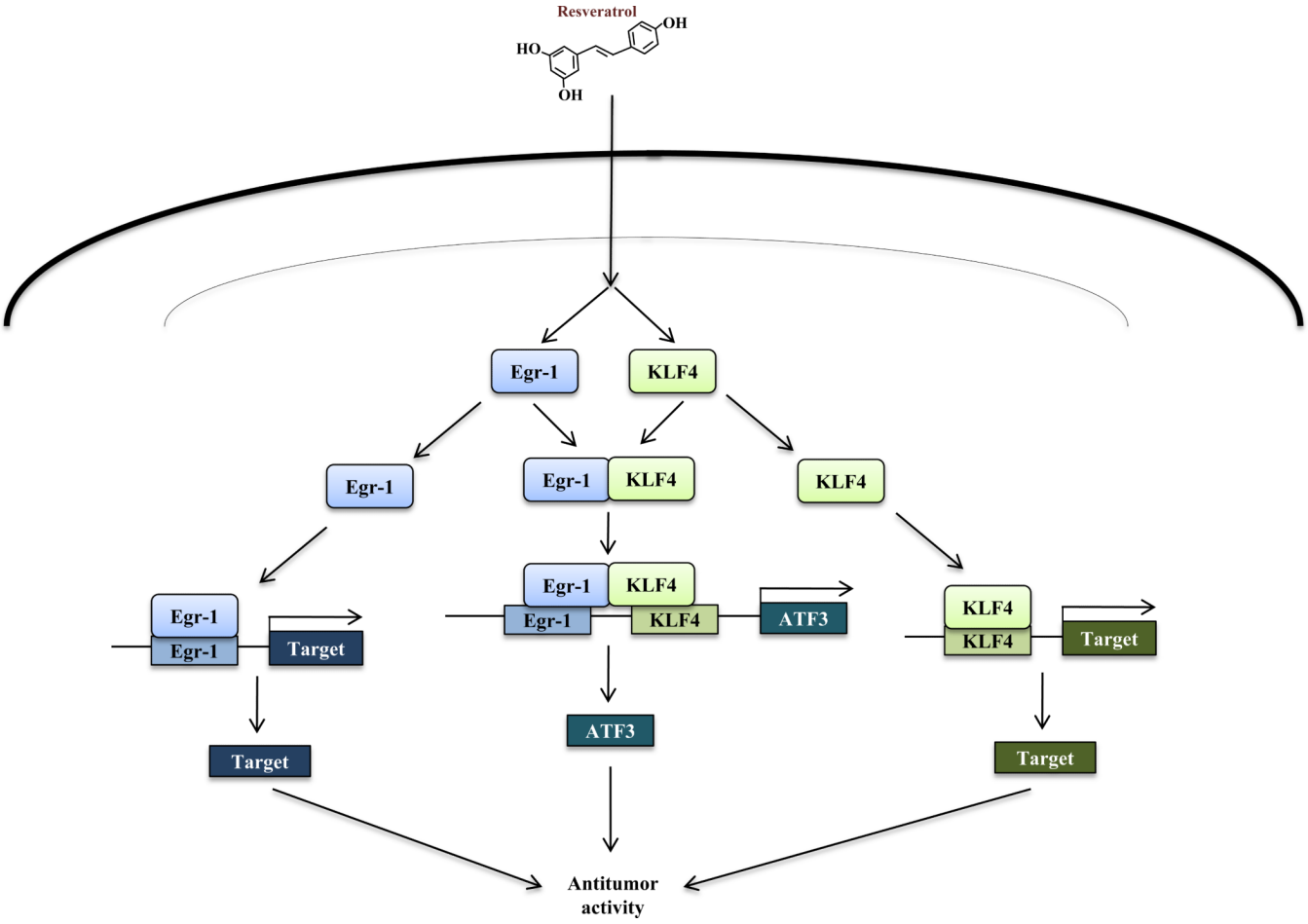

Fig. 4. Schematic diagram of ATF3-mediated resveratrol action in colorectal cancer

Resveratrol increases the expression of both Egr-1 and KLF4, facilitating their interaction. The Egr-1/KLF4 complex binds to its response elements on the *ATF3* promoter and mediates *ATF3* trans-activation. Increased ATF3 expression results in increase of antitumor activity (58). Alternatively, resveratrol-increased Egr-1 or KLF4 expression results in trans-activation of antitumor-related target genes.