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Multiple molecular Targets of Resveratrol: Anti-carcinogenic Mechanisms

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Abstract

Plant-derived polyphenolic compounds, such as the stilbene resveratrol (*trans*-3, 4', 5-trihydroxystilbene), have been identified as potent anti-cancer agents. Extensive *in vitro* studies revealed multiple intracellular targets of resveratrol, which affect cell growth, inflammation, apoptosis, angiogenesis, and invasion and metastasis. These include tumor suppressors p53 and Rb; cell cycle regulators, cyclins, CDKs, p21WAF1, p27KIP and INK and the checkpoint kinases ATM/ATR; transcription factors NF-κB, AP-1, c-Jun, and c-Fos; angiogenic and metastatic factors, VEGF and matrix metalloprotease 2/9; cyclooxygenases for inflammation; and apoptotic and survival regulators, Bax, Bak, PUMA, Noxa, TRAIL, APAF, survivin, Akt, Bcl-2 and Bcl-X_L. In addition to its well-documented anti-oxidant properties, there is increasing evidence that resveratrol exhibits pro-oxidant activity under certain experimental conditions, causing oxidative DNA damage that may lead to cell cycle arrest or apoptosis. This review summarizes *in vitro* mechanistic data available for resveratrol and discusses new potential anti-cancer targets and the anti-proliferative mechanisms of resveratrol.

Keywords

resveratrol; human cancer; cell cycle regulation; apoptosis; transcription factors; pro-oxidant; lysosome; growth signals

Introduction

Resveratrol (*trans*-3, 4', 5-trihydroxystilbene, $C_{14}H_{12}O_3$) is a plant-derived polyphenolic phytoalexin produced by the enzyme stilbene synthase in response to environmental stress such as vicissitudes in climate, exposure to ozone, sunlight and heavy metals, and infection by pathogenic microorganisms. Resveratrol exists in both *cis*- and *trans*-stereoisomeric forms.

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Exposure to heat and ultraviolet radiation can cause *trans*-resveratrol to isomerize to the *cis*-resveratrol. It is primarily found in the skin of grapes as well as in other fruits and plants, such as raspberries, blueberries, mulberries, Scots pine, Eastern white pine, and knotweed. Resveratrol has been shown to exhibit a wide range of health-promoting benefits for the coronary, neurological, hepatic, and cardiovascular systems [1,2]. It has been shown to inhibit inflammation, viral infection, oxidative stress, and platelet aggregation [3-5] and the growth of a variety of cancer cells [6]. The potent anti-cancer potential of resveratrol was recognized as early as 1997, when it was shown to block initiation, promotion, and progression of tumorigenesis induced by the polynuclear aromatic hydrocarbon dimethylbenz(a)anthracene (DMBA) [7]. Thereafter extensive studies have verified the cancer-preventing and anti-cancer properties of resveratrol in various murine models of human cancer, including skin cancer (both chemically and ultraviolet B-induced), gastric and colorectal cancer, lung cancer, breast cancer, prostate cancer, hepatoma, neuroblastoma, fibrosarcoma, pancreatic cancer, and leukemia [2, 8].

In the U.S. alone, almost 1.5 million new cases of invasive cancer were estimated to occur in 2007, as well as another 1 million new cases of non-melanoma skin cancer (basal cell and squamous cell carcinomas) (Cancer Factors and Figures 2007, American Cancer Society). Phytochemicals are among the most promising chemopreventive and treatment options for the management of cancer. The ideal characteristics that chemopreventive/therapeutic agents should possess include restoration of normal growth control to preneoplastic or malignant cells by modulating aberrant signaling pathways and/or inducing apoptosis; and targeting the multiple biochemical and physiological pathways of tumor development [9-12]. In this regard, resveratrol represents such an ideal molecule, due to its relatively low toxicity and capacity to target multiple signaling molecules that collectively promote cancer cell survival and tumor growth. The survival of cancer cells depends on their ability to adapt to changes in their microenvironment and to escape from the growth inhibitory effects of neighboring normal cells and to resist apoptosis and growth-inhibitory signals, leading to tissue invasion and metastasis. It is known that dysregulation of a number of molecules and signaling pathways has been identified as contributing to tumorigenesis. Some of these molecules include mutational activation of the oncogene Ras and deregulation of MYC by mutation or amplification; overexpression of AP-1 transcription factor components c-Fos and c-Jun; amplification, overexpression, or mutation of cell cycle regulator cyclins D/E and Cdks2/4; mutation of proapoptotic regulators Fas and Bax; mutation or deletion of the tumor suppressors p53, PTEN, and Rb; mutation of the DNA-damage response regulators Chk1/2 and ATM/ATR; mutation, amplification, or overexpression of survival kinase Akt; mutation of cell cycle inhibitors p21WAF1, p27KIP1, p14ARF, p16INK4A, and p15INK4B and translocation of anti-apoptotic Bcl-2. Numerous investigations demonstrated that resveratrol can modulate many if not most of the above-mentioned cancer targets (Table 1), suppressing cancer cell growth and/or inducing apoptosis, and even potentiates the apoptotic effects of cytokines, such as TRAIL, chemotherapeutic agents, and gamma radiation. This review discusses the anti-cancer mechanisms of resveratrol with respect to its molecular targets (collected through extensive data in human cell culture) and presents new targets for this promising natural anti-cancer compound and a new emerging view of resveratrol's mode of action.

I. Regulating Cell Cycle Progression

Cell cycle progression is tightly regulated by interaction between cyclin-dependent kinases (Cdk1, 2, 4, or 6), regulatory cyclin subunits (cyclin A, B, Ds, or E), and inhibitor proteins, such as p21WAF1 and p27KIP1 [13,14]. The coordinated activities of cyclin Ds/Cdk4/6, cyclin E/Cdk2, and cyclin A/Cdk2 are required for G1/S transition and progression through S phase, while Cdk1/cyclin A and B activities are required for entry into mitosis. Cyclin D1 is a rate-limiting activator for the G1/S transition, a critical cell-cycle checkpoint. The G1/S transition

requires the activation of the cyclin D/Cdk4/Cdk6 and cyclin E/Cdk2 complexes, which in turn phosphorylates the retinoblastoma protein (Rb). The subsequent dissociation of E2Fs from Rb activates a series of target genes that are required for entering S phase.

Cell cycle kinase activities are frequently upregulated in human cancers due to the overexpression of cyclins and Cdks, or the inactivation of the Cdk inhibitors. Specifically, deregulation of the cyclin D1-Rb axis is common in human cancers, as cyclin D1 accumulation is found in various types of human malignancies of breast, esophagus, liver, lung, skin, and affect cell cycle modulation perhaps its most extensively studied target. Resveratrol has been shown to modulate the major cell cycle mediators at micromolar concentrations, arresting cancer cells at the G1/S phase of the cell cycle. The anti-proliferative activity of resveratrol involves the induction of p21WAF1 and p27KIP1 and downregulation of cyclins D1/D2/E, Cdks 2/4/6, and hyperphosphorylated pRb [1,15,16]. In other cell types, resveratrol has been reported to arrest the cell cycle at the S-phase [17-19] as well as at the G2/M-phase, by inhibiting Cdk7 and p34Cdc2 kinases [20]. Resveratrol upregulates the p53 tumor suppressor protein [15] and its post-translational modification which may be related to its prooxidant stress response [21]. It induces the expression of p53-responsive genes (p21WAF1, p300/CBP, APAF1, and Bak) and causes Bcl2 downregulation [22]. In addition, p53-independent induction of p21WAF1 and subsequent cell cycle arrest in cells lacking wild-type p53 protein has been documented [1,15].

Resveratrol directly inhibits DNA synthesis by diminishing ribonucleotide reductase and DNA polymerase [23,24]. Resveratrol downregulated c-MYC in medulloblastomas in which 73% of tumor tissues expressed this oncogene and its downregulation was accompanied by S phase arrest [25]. Upstream of MYC, Cdk1 inhibition has recently been shown to induce rapid apoptosis in cells overexpressing MYC [26]. Cdk1 inhibition downregulates survivin, a known Cdk1 target required for the survival of cells overexpressing MYC. MYC-dependent apoptosis was observed *in vitro*, as well as in MYC-dependent mouse lymphoma and hepatoblastoma tumors [26]. In prostate cancer cells, resveratrol decreases cyclin B and Cdk1 expression and cyclin B/Cdk1 kinase activity in both androgen-sensitive and androgen-insensitive manners [27]. Lack of effective small-molecule inhibitors that selectively target the MYC pathway [26] prompted us to propose that resveratrol-mediated Cdk1 inhibition may be a useful approach for the treatment of human cancers with MYC overexpression. Using structure-activity relationship approaches, more effective analogs can be developed to treat these cancers.

Checkpoints play an important role in cell cycle progression. A critical target of checkpoint mechanisms is structurally altered DNA that occurs as a consequence of exposure to UV radiation or DNA damaging agents. Cells respond to DNA damage via sensors that activate checkpoint pathways and delay progression through the cell cycle at the G1, S, or G2 phases. Protein complexes containing several functional modules, such as ATM and ATR, sense DNA damage and signal downstream to promote cell cycle arrest, DNA repair or possibly apoptosis. ATM can phosphorylate p53 and triggers p53-dependent G1/S cell cycle arrest via p53 stabilization. Alternatively, ATM/ATR kinases can phosphorylate and activate the Chk protein kinase family (Chk1 and/or Chk2/Rad52/Cds1). Chk kinases phosphorylate and then inactivate cdc25 protein phosphatases. Cdc25 activity is required to activate both Cdk2 and Cdc2 by dephosphorylating the tyrosine 15 residue on cdk molecules. In contrast to the p53-dependent pathway, this p53-independent checkpoint is rapid and operates post-translationally, leading to the inhibition of Cdk2 by tyrosine phosphorylation. Resveratrol has been shown to induce S-phase cell cycle arrest through the ATM/Chk pathway in human malignant B cells [28] and cause Cdc2-tyr15 phosphorylation via activation of the ATM/ATR-Chk1/2-Cdc25C pathway in ovarian cancer cells, whereas only marginal S-phase arrest is observed in normal human foreskin fibroblasts [29]. Taken together, the anti-proliferative activity of resveratrol involves

the differential regulation of the multiple cell cycle targets, which may be dependent on both concentrations of resveratrol and characteristics of target cells [15,18,19,30,31].

II. Regulating Apoptosis and survival Pathways

The primary growth-inhibitory effects of resveratrol are mediated via both p53-dependent and p53-independent upregulation of p21WAF1 and downregulation of key cell cycle activators. A number of studies have demonstrated that resveratrol-induced growth arrest is followed by apoptotic cell death and that it directly interferes with cell survival by the modulation of apoptotic and survival pathway genes. Apoptosis is regulated by a complex network of pro-apoptotic and anti-apoptotic proteins. The apoptotic signals can be initiated by external stimuli/ligands and by cellular stress caused by gamma/UV radiation and cytotoxic drugs, leading to altered mitochondrial permeability. As a consequence of alterations in mitochondria permeability pore transition, release of cytochrome *c* into the cytoplasm occurs where it can bind to and induce conformational change of APAF-1, resulting in the formation of the "apoptosome" complexes. Apoptosomes recruit and activate caspase 9, which in turn activates the effector caspases 3, 6, and 7. Caspase activation is tightly regulated by the inhibitor of apoptosis proteins (IAP), which include NAIP, cIAP1, cIAP2, XIAP, and survivin. IAP binds to caspases and antagonizes their activity. SMAC, the second mitochondria-derived activator of caspase, also known as DIABLO, is released into the cytoplasm and binds and neutralizes the IAPs; this restores caspase activity and induces apoptosis [32]. Survivin prevents Smac/DIABLO release from mitochondria in certain cancer cells [33]. Mitochondrial release of cytochrome C is further regulated by Bcl2 family proteins. Bcl2-like anti-apoptotic proteins reside in the outer mitochondrial membrane and inhibit cytochrome C release, while proapoptotic Bax, BID, and BIM translocate to mitochondria to facilitate apoptosis. p53 induces a number of mitochondria-mediated apoptotic genes, such as Bax, Noxa, PUMA, and BID, and represses the anti-apoptotic Bcl2 and CIAPs. Induction of phosphatase and tensin homolog deleted on chromosome-10 (PTEN), APAF-1, and p53-regulated apoptosis-inducing protein-1 (p53AIP1) may also contribute to apoptosis [34]. Resveratrol-mediated apoptosis has been associated with p53 activation in various human cancer cells [35,36]. It induces the expression of pro-apoptotic Bax, Bak, PUMA, Noxa, and Bim, and inhibits the expression of anti-apoptotic Bcl2, Bcl-XL, and Mcl-1, directly affecting the mitochondrial death pathway [36]. Consequently, Bcl2 overexpression has been shown to suppress resveratrol-induced caspase-3 activation and apoptosis [37]. In thyroid cancer cells, resveratrol-induced apoptosis has also been associated with the accumulation of p53 (ser15) phosphorylation and non-steroidal anti-inflammatory (NSAID) drug-activated gene-1(NAG-1) with pro-apoptotic and anti-tumorigenic activities. Interestingly, the p53 binding sites within the promoter region of NAG-1 have been shown to play a pivotal role in controlling the effects of resveratrol on NAG-1 expression [38].

Resveratrol-mediated apoptosis also occurs via the death receptor Fas/CD95/APO-1 [39]. Fas/CD95/APO-1/DR2 and TRAILR are death receptor family members, that activate a death-signaling cascade after binding to corresponding ligands [40,41]. The cytoplasmic domain (death domain, DD) of Fas interacts with adaptor proteins, such as Fas-associated via death domain (FADD) which in turn recruits procaspase 8. Active caspase 8 can directly cleave caspase 3 and FLICE-inhibitory protein (FLIP) is known to inhibit caspase-8 activation [42, 43]. Amplification of the death signal can also occur in certain cells via the engagement of the mitochondrial pathway of caspase activation. Proteolytically cleaved, active procaspase-8 cleaves BH3 interacting domain death agonist (BID), resulting in tBID (truncated BID), which can inactivate Bcl2. Inhibition of Bcl2 in the mitochondrial membrane releases cytochrome C, which binds to APAF1 and caspase-9 which can activate procaspase-3. Resveratrol can redistribute FAS/CD95 into lipid rafts in a ligand-independent way, enhancing the efficacy of signaling by FAS/CD95 and other death receptors in colon cancer cells [44]. It also induces

the redistribution of FAS/CD95 and other death receptors in lipid rafts, and sensitizes cells to death receptor agonists [45]. A similar synergy has been reported between resveratrol and TRAIL [46]. Resveratrol enhanced TRAIL-induced apoptosis through G1 cell cycle arrest and survivin depletion [47]. Furthermore, resveratrol was shown to overcome the chemoresistance of human multiple myeloma cells and potentiated the apoptotic effects of bortezomib and thalidomide through the suppression of NF-κB and STAT3, which in turn lead to the downregulation of anti-apoptotic genes, cyclin D1, cIAP-2, XIAP, survivin, Bcl-2, Bcl-xL, Bfl-1/A1, TRAF2, and Akt and increased Bax/caspase-3-associated apoptosis [48]. In MCF7 human breast cancer cells, the phosphorylation of Akt was significantly reduced which is followed by decreased pro-caspase-9 activation [49]. In LNCaP and PC-3 prostate cancer cells, resveratrol can inhibit AR (androgen receptor)-, and ER (estrogen receptor) alpha-dependent PI3K phosphoinositide-3-kinase activities, respectively [50].

The sphingomyelin pathway responds to diverse environmental stresses such as UV radiation, heat shock, oxidative stress, cytokines (TNF-α and IL-lβ), and anti-cancer drugs and is involved in inflammation, cell cycle arrest, apoptosis, and stress [51,52]. Ceramide is the second messenger in this pathway, generated either by the hydrolysis of sphingomyelin or by *de novo* synthesis. Ceramide induces apoptosis through c-Jun N-terminal kinase (JNK) and stress-activated protein kinase (SAPK) and promotes the dephosphorylation of Bcl2, Bax, and Bad. It also activates caspase-3. In addition, ceramide regulates cathepsin-D, binding directly to PLA2 (phospholipase-A2), and may induce apoptosis [52]. The anti-proliferative effects of resveratrol correlate with a dose-dependent increase in *de novo* ceramide biosynthesis and subsequent inhibition of c-MYC and ODC in colon cancer cells [53]. Androgen-independent human prostate cancer cells, DU145, are resistant to ionizing radiation-induced cell death, but become sensitized to apoptosis with prior resveratrol treatment due to ceramide accumulation [54]. In metastatic breast cancer cells, ceramide mediates the anti-cancer effects of resveratrol [55]. Resveratrol also promotes the accumulation of mature cathepsin-D [56].

III. Inhibition of Tumor Invasion and Angiogenesis

The expression of endopeptidases and matrix metalloproteinases (MMPs) correlates with proteolytic degradation of the extracellular matrix and tumor metastasis, followed by angiogenesis, to sustain rapid growth [57]. The matrix metalloproteinases, particularly type IV collagenases MMP-2 and MMP-9, and the angiogenesis process, are attractive pharmaceutical targets for the treatment of cancer. MMP-2 and MMP-9 play an important role in the degradation of type IV collagen, which is a major component of the basement membrane. The expression of MMP-2 and MMP-9 is associated with metastasis of various human cancers [58-63]. MMP-9 expression is regulated by AP-1, and possibly by NF-κB and Sp1, as the human MMP-9 promoter contains *cis*-acting regulatory elements for these transcription factors [60]. Resveratrol has been shown to directly inhibit the growth of human umbilical endothelial cells (HUVECs) by decreasing the gelatinolytic activities of MMP-2, and to inhibit endothelial cell attachment to the basement membrane components fibronectin and laminin [64]. The migration of bovine aorta endothelial cells and the tube formation of vascular endothelial cells is inhibited by resveratrol [65] and the constitutive expression of MMP-2 and -9 proteins and their gelatinolytic activity are suppressed in multiple myeloma cells [66]. Resveratrol also inhibits DMBA-induced MMP-9 expression by suppressing NF-κB DNA-binding activity [67] and the activation of AP-1 [68]. Moreover, the suppression of PMA (phorbol myristate acetate)-induced MMP-9 expression by resveratrol results JNK inhibition and protein kinase C (PKC)-δ activation [69].

The characteristic features of angiogenesis are the loss of contact between endothelial cells in the parent vessel and the migration of endothelial cells from pre-existing capillaries [70,71]. Vascular endothelial growth factor (VEGF), which is a heparin-binding homodimeric

glycoprotein, interacts with the endothelial-specific receptor tyrosine kinases (VEGFR) [72]. VEGF is crucial for angiogenesis and tumor growth, as it is involved in blood vessel development. VEGF/VEGFR-signaling is known to target PI3K, which in turn activates Akt, leading to endothelial cell survival [73]. PLC-gamma and Src are also activated downstream of VEGF/VEGFR, and VEGF promotes Ras-independent induction of the Raf1>MEK1/2>ERK1/2<cPLA2, which in turn may regulate prostaglandin production [73]. In breast cancer cells, a significant decrease in extracellular levels of VEGF has been associated with apoptosis following resveratrol treatment [74]. In addition, resveratrol has been shown to suppress the invasiveness of cancer cells through inhibition of hypoxia-mediated activation of ERK 1/2 and Akt, leading to a marked decrease in hypoxia-induced HIF-1α protein accumulation and VEGF [75,76]. Vascular endothelial-specific adhesion protein, VE-cadherin, and its anchoring partner, β-catenin complex, are considered important in regulating cell junction stability. The angiogenic VEGF-mediated increase of tyrosine phosphorylation of VEGFR-2 and VE-cadherin has been associated with cell migration and tumor formation [77]. Resveratrol-mediated disruption of ROS-dependent Src kinase activation and subsequent VE-cadherin tyrosine phosphorylation have been shown to be critical for the inhibition of VEGF-induced angiogenesis [78]. Resveratrol inhibits the growth of rat gliomas through the suppression of angiogenesis [79,80].

IV. Inflammation in Cancer: COX-2 and its Regulation

Tissue inflammation is emerging as a significant epigenetic factor in the initiation/progression stages of cancer development by inducing oxidative damage and promoting cell growth [81]. Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme that catalyzes the conversion of free arachidonic acid to prostaglandins. It is induced in many cells by inflammatory mediators and various stimuli, including mitogens, oncogenes, tumor promoters and growth factors. Prostaglandins can stimulate tumor cell proliferation, promote angiogenesis, and suppress apoptosis all of which promote malignancy [82-84]. Aberrant COX-2 expression is found in both premalignant and malignant conditions associated with various types of human cancers, including colon, prostate, liver, pancreas, breast, lung, bladder, and skin cancer, suggesting that proinflammtory mediators promote carcinogenesis [82,85-87]. COX-2-dependent reactions generate ROS during the conversion of arachidonic acid to prostaglandin G(2), causing direct oxidative damage to DNA. A significant increase in the amount of 8-oxo-2'-deoxyguanosine, a marker of oxidative DNA damage, has been observed in the presence of COX-2 [82,88]. Resveratrol directly inhibits COX-2 activity [89], and blocks TPA-induced NF-κB activation and COX-2 expression in mouse skin *in vivo* [90]. Hydroxylated resveratrol analogs selectively inhibit COX-2 activity [91]. As a mechanism upstream to COX-2 inhibition, resveratrol was shown to regulate MKP5, a member of the dual-specificity MKP family of phosphatases that dephosphorylate mitogen-activated protein kinases (MAPKs). MKP5 specifically dephosphorylates the stress-activated protein kinases p38 and JNK thereby inhibiting them [92,93]. p38 is known to mediate pro-inflammatory responses in prostate cancer, and p38 inhibition leads to decreased activation of NF-κB, reduced COX-2 expression and diminished release of pro-inflammatory cytokines. MKP5 has recently been identified as a potent anti-inflammatory mediator, as MKP5 overexpression decreased cytokine-induced NF-κB activation, COX-2, IL-6, and IL-8 in normal prostatic epithelial cells by inhibiting p38 MAPK. Resveratrol induces MKP5 in a number of prostate cancer cells [94]. Additionally, resveratrol-induced apoptosis in human breast cancer cells, MCF-7 and MDA-MB-231, occurs concomitant with ERK1/2 and AP-1-dependent nuclear accumulation of COX-2, which co-localized with Ser(15)-phosphorylated p53 and p300, a co-activator for p53-dependent gene expression [95]. The interaction of COX-2/p53/p300 and subsequent resveratrol-induced apoptosis were inhibited by abrogating ERK1/2 activity. These data suggest that resveratrol may affect cancer progression by acting on inflammation-related proteins in a cell context-dependent manner.

V. Modulation of Transcription Factors

NF-κB

The transcription factor nuclear factor-kappa B (NF-κB) is implicated in various cellular processes, including immune and stress responses, inflammation, apoptosis, and regulation of cell growth. Aberrant and sustained NF-κB activity has been implicated in various stages of tumorigenesis and is found in a number of cancers. NF-κB is composed of homo- and heterodimeric complexes, consisting of p50, p65/RelA, c-Rel, p52, and RelB. Each complex exhibits different DNA-binding affinity and transactivation potential. In its inactive state, NF-κB is sequestered in the cytoplasm, bound by the family of inhibitor proteins, IκBs. Following exposure to external stimuli such as mitogens, cytokines, UV, ionizing radiation, and bacterial toxins, IκB kinase (IKK) phosphorylates IκBα, leading to ubiquitination-dependent degradation of IκBα. Dissociation of IκBα from NF-κB allows nuclear translocation of the activated free NF-κB dimer, where it binds to the specific *cis*-acting sequence in the promoter of target genes, such as COX-2 and MMP. NF-κB is also activated by oncogenes such as Ras and Bcr-Abl, growth factors, and by other kinases, such as Akt and p38 MAPK. p38 MAPK-dependent phosphorylation of histone H3 is demonstrated in the recruitment of NF-κB to a selected chromatin. Resveratrol can suppress IKK phosphorylation and can block the subsequent degradation of IκBα, thereby inhibiting the activation of NF-κB in TPA-stimulated mouse skin. Pretreatment with resveratrol also inhibited TPA-induced phosphorylation of p65 and its interaction with CBP/p300, as well as phosphorylation of p38 mitogen-activated protein (MAP) kinase [90]. Resveratrol-mediated blockade of NF-κB activation in various cancer cells, by different stimuli such as TNF, PMA, LPS, H_2O_2, okadaic acid, or ceramide can be observed [96]. Resveratrol can also downregulates the expression of NF-κB-regulated genes, including interleukin-6, Bcl-2, Bcl-xL, XIAP, c-IAP, vascular endothelial growth factor (VEGF), and matrix metalloproteinase-9 (MMP-9) [97]. Resveratrol-mediated inhibition of NF-κB and MMP-9 activities in breast cancer cells blocks their migratory potentials [98].

AP-1

The transcription factor AP-1 can be produced by a number of different dimeric combinations of the Jun (c-Jun, JunB and JunD) and Fos (c-Fos, FosB, Fra-1 and Fra-2) families and Jun dimerization partners (JDP1 and JDP2) and activating transcription factor (ATF2, LRF1/ATF3 and B-ATF) subfamilies [99]. AP-1 plays important roles in the proliferation of initiated cells as well as in the metastasis of tumor cells, and induces COX-2, urokinase-type plasminogen activator, Fos, MMP-9, cyclin D1, and VEGF [100]. Resveratrol can inhibit c-Fos mRNA expression in TPA-treated murine skin [101] and suppresses AP-1 DNA binding affinity [99]. Downregulation of c-Jun and suppressed AP-1 activity by resveratrol involves inhibition of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK)1 > ERK1/2 signaling [1].

P53

Resveratrol can regulate the transcriptional activity of p53. ERK and p38 MAPK mediate this resveratrol-induced activation of p53, which may be followed by the manifestation of apoptosis, involving phosphorylation of p53 at serine 15 [102]. In papillary thyroid carcinoma and follicular thyroid carcinoma cells, Ras-MAPK signal transduction pathway regulates p53-dependent apoptosis [103]. As discussed earlier, resveratrol is a potent inducer of NAG-1, which is a downstream of p53 and TGF-β superfamily protein. The ectopic expression of NAG-1 results in the reduction of colony formation and the induction of apoptosis, and is mediated in a p53-dependent manner in various cancer cells [38].

VI. Lysosomal Cathepsin D as a Novel Target

Cathepsin D, an aspartic endoprotease, is ubiquitously expressed in lysosomes. Cathepsin D is overexpressed and hyper-secreted by epithelial breast cancer cells, possibly through extracellular interaction with a yet-unknown cell surface membrane receptors, and serves as a marker for poor prognosis. It is regulated by estrogens and certain growth factors, including IGF1 and EGF, in ER-positive breast cancer cells. Both estrogen and growth factors induce accumulation of cathepsin D protein and mRNA [104]. Cathepsin D, however, is also a key mediator of apoptosis induced by stimuli such as IFN-gamma, FAS/APO, TNFα, oxidative stress, and DNA-damaging agents. Depending on the experimental cell model and stimuli, its role in apoptosis involves the cytosolic translocation of mature lysosomal cathepsin D, leading to the mitochondrial release of cytochrome *c* and subsequent activation of caspases 3 and 9 [104]. In addition, the cytosolic relocalization of cathepsin D can trigger Bax activation, followed by the selective release of mitochondrial apoptosis-inducing factor (AIF) [105]. Recently, resveratrol-induced cell death was shown to involve lysosomal proteolytic pathways, in which lysosomal cathepsin D acts upstream of caspase activation. The inhibition of cathepsin D prevents Bax oligomerization, mitochondrial membrane permeabilization, cytochrome c release, and caspase 3 activation [56]. Resveratrol appears to manifest biphasic effects in a dose-dependent manner. It increases cathepsin D and IGF-II secretion in ER+, but not in ER −, breast cancer cells at lower concentrations (10^{-6} M), whereas resveratrol treatment at a higher concentration (10^{-4} M) inhibits cathepsin D in these cells [106].

VII. Adenosine Monophosphate (AMP)-Activated Protein Kinase

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is an eukaryotic heterotrimeric serine/threonine kinase that senses nutritional and environmental stresses and functions as a metabolic master switch [107]. AMPK phosphorylates and regulates *in vivo* hydroxymethylglutaryl-CoA reductase and acetyl-CoA carboxylase, key regulatory enzymes of sterol synthesis and fatty acid synthesis, respectively [108]. AMPK is activated by high AMP and low ATP. Energy depletion leads to the kinase-cascade activation of LKB1>AMPK>TSC1/2 >mTOR pathway, in which a blockade of LKB1 activity abolishes AMPK activation in response to different stimuli [109]. AMPK is also implicated in cancer development and control, and is emerging as a potential anti-tumor target molecule. Mutational inactivation of LKB1 leads to Peutz-Jeghers syndrome, a dominantly inherited cancer susceptibility gene humans [109]. In a recent study, a combinatorial treatment of resveratrol and etoposide induces apoptosis in etoposide-resistant colon cancer cells by activating AMPK signaling cascade. In this study, ROS was found to be upstream of AMPK activation [107].

VIII. Pro-oxidant Activity of Resveratrol

Reactive Oxygen Species (ROS), such as superoxide and hydrogen peroxide, are by-products of normal aerobic metabolism, which at low levels, play important roles in cell signaling processes. At higher concentrations, ROS induce apoptosis. ROS has been shown to mediate post-translational modifications of p53 and induces disruption of mitochondrial membrane permeability and apoptotic DNA fragmentation [110,111].

Resveratrol effectively scavenges superoxide and peroxynitrite radicals generated from enzymatic and nonenzymatic systems and affords protection against DNA damage caused by these ROS [100]. Resveratrol may also exhibit pro-oxidant properties, catalyzing cellular DNA degradation in the presence of transition metal ions such as copper [112]. In this respect, resveratrol was shown to catalyze the reduction of Cu(II) to Cu(I), which is accompanied by the formation of oxidized product(s) of resveratrol [113,114]. Resveratrol also augments H_2O_2/Cu(II)-induced DNA strand breaks. In independent studies, resveratrol, but not genistein, manifested DNA strand breaks involving redox cycling Cu(II)/Cu(I) and H_2O_2 [115]. Similar

to many plant polyphenols, resveratrol may mobilize endogenous copper, such as chromatin-bound copper, to manifest its pro-oxidant activity [113,116]. Elevated copper (Cu) levels occur in hepatocellular carcinoma cells as compared to normal cells [117] and copper metabolism is upregulated in many other tumors [118]. Therefore, these cancer cells may be more susceptible to ROS generation by resveratrol [119]. Resveratrol decreases mitochondrial membrane potential and increases ROS generation [98]. In this respect, resveratrol has also been shown to enhance UVA-induced DNA damage in HaCat keratinocytes [120]. Furthermore, chronic resveratrol treatment employing its subapoptotic concentrations increases hydrogen peroxide and superoxide anion levels and causes ATM-dependent senescence in p53-positive tumor cells [121]. ROS-dependent senescence-like growth arrest in resveratrol-treated tumor cells involves activation of p38 MAPK, p53 and p21WAF1 [121].

Conclusion

Similar to several dietary compounds, such as epigallocatechin gallate (EGCG), quercetin, genistein, and curcumin, resveratrol shows great potential for the prevention of human cancers. Intense mechanistic and preclinical studies indicate that resveratrol is capable of preventing and delaying malignant growth both *in vitro* and *in vivo*. The pharmacokinetic, pharmacodynamic and safety properties of resveratrol are currently being investigated in early clinical phase I trials. A non-randomized, open-label study is being conducted in a group of patients with resectable colorectal cancer, using COX-2 expression and Ki67 labeling index as biomarkers. Based on limited biological data in humans, resveratrol is considered pharmacologically quite safe. Currently, structural analogues of resveratrol with improved bioavailability are being pursued as potential cancer therapeutic agents [6]. Knowing that resveratrol regulates multiple molecular targets and signaling pathways, it will be important to determine exactly how resveratrol regulates and influences these intracellular targets and pathways. It is likely that resveratrol acts as an endogenous signaling molecule, or act through the generation of common signaling mediators such as ROS or both. Further investigation will clarify some of these possibilities and may be translated to develop this promising bioactive compound for human cancer prevention and therapy.

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Abbreviations

Akt1
v-Akt murine thymoma viral oncogene homolog-1

AP-1
activator protein 1

BAK
Bcl2 antagonist/killer

APAF1
apoptotic protease activating factor-1

BAX
Bcl2 associated X protein

BID
BH3 interacting domain death agonist

Bcl2
B-cell CLL/lymphoma-2

BIM
Bcl2-interacting protein

COX
cyclooxygenase

DIABLO
direct IAP binding protein with low pI

ERK1/2
extracellular signal-regulated kinase 1/2

FADD
Fas-associated via death domain

FLICE
FADD-like ice

FLIP
FLICE-inhibitory protein

HUVEC
human umbilical vein endothelial cell

IAP
inhibitor of apoptosis proteins

JNK
c-Jun NH2-terminal kinases

MMP1
matrix metalloproteinase-1

NAG-1
non-steroidal anti-inflammatory (NSAID) drug-activated gene-1

NF-κB
nuclear factor kappa B

Noxa1
NADPH oxidase activator 1

p53AIP1
p53-regulated apoptosis-inducing Protein-1

PI3K
phosphoinositide-3-kinase

PLC-gamma
phospholipase-C Gamma

PMA
phorbol myristate acetate

PTEN
phosphatase and tensin homolog deleted on chromosome-10

PUMA
p53-upregulated modulator of apoptosis

Rb
retinoblastoma protein

ROS
reactive oxygen species

Smac
second mitochondria-derived activator of caspase

STAT
signal transducers and activators of transcription

TPA
tumor promoter 12-O-tetradecanoylphorbol-13-acetate

TRAIL
TNF-related apoptosis-inducing ligand

TRAILR
TNF-related apoptosis-inducing ligand receptor

VEGF
vascular endothelial growth factor

XIAP
X-linked inhibitor of apoptosis protein

Table-1

Effects of resveratrol on various human cancer cells.

TUMOR MODELS	CELL LINES USED	MOLECULAR TARGETS	CELLULAR EFFECT(S)	REFERENCES
Breast cancer	T47D MDA-MB-231 MDA-MB-468 MCF-7	p53, PTEN, p27, ROS, NO, QR, p21	Apoptosis	(Alkhalaf, 2007; Kim *et al*., 2004; Kotha *et al*., 2006; Lanzilli *et al*., 2006; Lee and Safe, 2001; Pozo-Guisado *et al*., 2002; Pozo-Guisado *et al*., 2005; Waite *et al*., 2005)
		p70S6K, ppS6RP, Src-Stat3, pAkt, Bcl-2, NF-κB, calpain, MMP-9, cyclin D, Cdk4, ribonucleotide reductase, CYP1A1, telomerase	Growth arrest Cell migration	
Prostate cancer	LNCap PC-3 DU145 Colo-357 LAPC-4	Caspases 3/9, p53, p21, p27, Bax, Bak, Bid, Bad, MKP5	Apoptosis, G0/G1-arrest	(Awad *et al*., 2005; Aziz *et al*., 2006; Benitez *et al*., 2007a; Kotha *et al*., 2006; Nonn *et al*., 2007)
		PI3K, pAKT, cyclins B/D1/E, Cdk1/4, Bcl-2, Src-Stat3, ROS	Proliferation rate, cell viability	
Colon cancer	HT-29 DLD1 HCT116	AMPK, ROS, cathepsin D, caspase-2, cytochrome c, ATF3	Apoptosis, lysosome leakage, G2-arrest	(Bottone *et al*., 2005; Hwang *et al*., 2007; Liang *et al*., 2003; Mohan *et al*., 2006; Trincheri *et al*., 2007)
		Cdk7, p34Cdc2	Cell growth	
Pancreatic cancer	CD18 S2-013 panc-1	MIC-1, cytochrome C, caspase-3	apoptosis	(Golkar *et al*., 2007; Kotha *et al*., 2006; Mouria *et al*., 2002)
		Src-Stat3, NF-κB	Cell growth	
Leukemia	HL-60		Apoptosis, nuclear size, granularity	(Quiney *et al*., 2004; Stervbo *et al*., 2006)
		NO	Cell growth	
Hepatoma	HepG2		Apoptosis, nuclear size, granularity	(Stervbo *et al*., 2006)
			Cell growth	
B-cell Lymphoma	LY1 LY8 LY18	p27, p53, CD69	Apoptosis, G0/G1-arrest	(Faber and Chiles, 2006; Faber *et al*., 2006)
		BCL6, Myc, pAKT, pp70S6K	glycolysis	
Osteosarcoma	SJSA1	pERK1/2, pp53(Ser15)	Apoptosis	(Alkhalaf and Jaffal, 2006)
			Cell growth	
Squamous cell carcinoma	A431	p21, p27	G0/G1-arrest	(Adhami *et al*., 2001; Ahmad *et al*., 2001; Kim *et al*., 2006; Zhang *et al*., 2005)
		Cyclins A/E/D1/D2 Cdk2/4/6, pRb, MEK1, pERK1/2, c-Jun, AP-1, HIF-1α, VEGF, Akt, E2F1-5, DP1/2		
Multiple Myeloma	RPMI8226 OPM-2 U266 KM3	c-fms, CD14, CD11a, 1,25(OH)2D3 nuclear receptor, Bax, Apaf-1	apoptosis	(Boissy *et al*., 2005; Jazirehi and Bonavida, 2004; Sun *et al*., 2006b)
		Cathepsin K, RANK, NFATc1, NF-κB nuclear translocation, Bcl-2, Bcl-x (L), XIAP, Mcl-1, MMP-2, MMP-9		
Rhabdomyosarcoma			S/G2-arrest	(Chow *et al*., 2005)
		Cyclin B		

TUMOR MODELS	CELL LINES USED	MOLECULAR TARGETS	CELLULAR EFFECT(S)	REFERENCES
Ovarian Carcinoma	Ovcar-3 A2780/CP70 CaOV3 ES-2 TOV112D A1947	pCdc2(tyr15), ATM/ATR, chk1/2, pCdc25C, pH2A.X(ser139),	S-arrest, autophagocytic death	(Cao *et al*., 2004; Opipari *et al*., 2004; Tyagi *et al*., 2005)
		Akt, HIF-1α, VEGF		
Medulloblastoma	UW228-2 UW228-3	CYP1A1	Apoptosis, differentiation	(Liu *et al*., 2004; Zhang *et al*., 2006)
		CYP1B1,c-Myc		
Acute myeloid leukemia	OCIM2 OCI/AML3		S-arrest, apoptosis	(Estrov *et al*., 2003)
		IL-1β, NF-κB		
Thyroid cancer	PTC FTC	P53, p53(ser15), c-fos, c-jun, p21	apoptosis	(Shih *et al*., 2002)
Gastric adenocarcinomas	KATO-III RF-1		G0/G1-arrest, apoptosis	(Atten *et al*., 2001)
		PKC, PKCα	DNA synthesis	

Upregulation and increased activities of molecular targets are represented in the upper rows, and inhibition and decreased activities in the bottom rows for each tumor model.