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Review

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, Bcl2 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.

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. 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 US 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

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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 pro-apoptotic 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[1]; mutation of cell cycle inhibitors p21WAF1, p27KIP1, p14ARF, p16INK4A, and p15INK4B and translocation of anti-apoptotic Bcl2. 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.

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].

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

[1] *Abbreviations used:* 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.