Direct molecular targets of resveratrol: identifying key interactions to unlock complex mechanisms

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To truly understand the mechanisms through which resveratrol exerts its biological effects, the key direct interactions between resveratrol and its target biomolecules must be identified. With an increasing number of biochemical tools to measure and quantify direct physical interactions between biomolecules, there have been around 20 proteins identified as having a specific affinity to resveratrol to date. Resveratrol has been described as a promiscuous molecule, and one would expect it to bind with numerous proteins, which would help explain why resveratrol appears to have so many health benefits and has been shown to act upon various different pathways related to a diverse range of conditions. The aim of this review is to present the direct protein targets of resveratrol that are currently known and highlight the consequences of direct binding and the methods used to identify the nature of these interactions.

Keywords: resveratrol; mechanism; protein targets; inflammation; cancer; cardiovascular

Introduction

Since the revelation that resveratrol might have anticancer properties,1 there have been numerous publications investigating the effects of resveratrol on various models of disease, including human clinical trials. There is little doubt that resveratrol exhibits multiple beneficial effects against diverse diseases and conditions in vitro and in vivo animal models, but the conclusive proof that resveratrol can live up to the claims in humans has not yet been demonstrated. The scale and breadth of research on resveratrol in the context of the treatment or prevention of disease cover cancer,2 cardiovascular disease,3,4 antiaging,5–7 metabolic syndrome,7,8 bone health,9 eye health,10 longevity,11,12 and Alzheimer’s disease13,14 and have provided a plethora of suggested mechanisms of action, as described in several comprehensive review articles on the subject.4,6,13–19

Mechanisms of action

Depending on the condition/disease studied and the model used, there have been a number of processes and molecular pathways shown to be affected by resveratrol, including apoptosis, senescence, autophagy, cell metabolism and proliferation, redox status, a wide variety of inflammatory and cell signaling pathways, and nitric oxide synthesis. The potential health benefits and related pathways actually affected in humans exposed to or treated with resveratrol are not yet known, but the most studied candidates to date, largely using preclinical systems, are anti-inflammatory mechanisms, cell cycle and programmed cell death pathways, calorie restriction mimetic via energy-sensing metabolic regulators, and resveratrol’s much publicized antioxidant properties. Other modes of action have been reported, including effects on mammalian target of rapamycin (mTOR) signaling,20 miRNA modulation,21,22 AMP-activated protein kinase (AMPK) activation,23 NF-κB activation,24 nitric oxide production,4 histone deacetylase (HDAC) inhibition,25 cytochrome P450 inhibition,26 and even prooxidant properties.27 Despite the wealth of information available, a greater understanding of resveratrol’s mechanisms of action at clinically achievable low concentrations, particularly the key events underlying efficacy, rather than collateral effects, is still required. This is necessary not only for

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Direct targets of resveratrol and subsequent mechanism-driven biomarkers. Direct binding to a target protein can alter its function and affect downstream pathways. Changes in levels of biomolecules such as proteins, nucleic acids, or metabolites then act as biomarkers of the effects of resveratrol.

**Direct interactions**

Table 1 summarizes the biomolecules that have been shown to directly interact with resveratrol. Only targets that have been confirmed by direct means have been included and, where available, the strength of the interaction or potency of the inhibition/activation is discussed. Figure 2 illustrates the range of mechanisms/pathways that are affected by direct binding of resveratrol to target proteins and can be divided into five rather broad subject areas: inflammation, metabolism, cell signaling, the cell cycle, and posttranslational modification.

**Inflammation targets**

Because chronic conditions such as cancer, cardiovascular disease, and metabolic syndrome are inexorably linked to inflammation, resveratrol’s effect on cyclooxygenase (COX) expression and activity, along with the related prostanoid levels, has been a subject of investigation. Resveratrol exerts anti-inflammatory effects in a number of *in vitro* and *in vivo* models and it has been shown to directly inhibit COX-1 and COX-2, but also to down-regulate the expression of COX-2, which is the inducible form of the enzyme and is most convincingly linked to chronic disease. COX inhibition has been demonstrated to reduce tumor burden and increase survival in preclinical mouse models of breast and colon cancer, and accumulating evidence indicates that the world’s oldest dynamic/calorimetric techniques such as surface plasmon resonance, capillary electrophoresis, and thermophoresis; (2) structural/spectroscopic methods including X-ray crystallography, 2D nuclear magnetic resonance spectroscopy, circular dichroism, mass spectrometry, and fluorescent assays; (3) chemical proteomics, such as affinity purification and tagged-ligand approaches; and (4) virtual/“in silico” methods used to predict binding on the basis of molecular modeling. Ideally, a combination of methods should be used to confirm and characterize the nature of the interaction, be it inhibition/activation, stabilizing/destabilizing, or conformational change leading to alteration of the normal properties of the protein. Subsequent systemic effects, altered pathways, and biomarkers from *in vitro* and *in vivo* models are no less important, and are vital to exploring the mechanisms engaged by this simple molecule with complex actions.
Table 1. Direct targets of resveratrol

<table>
<thead>
<tr>
<th>Molecular target</th>
<th>Method</th>
<th>Observation</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Cyclooxygenase-1</td>
<td>Enz Inhib, Xray</td>
<td>IC50 = 0.535 µM</td>
<td>28,33</td>
</tr>
<tr>
<td>Cyclooxygenase-2</td>
<td>Chem Prot, Enz Inhib</td>
<td>IC50 = 0.996 µM</td>
<td>28,29</td>
</tr>
<tr>
<td>Fatty acid synthase</td>
<td>Enz Inhib</td>
<td>IC50 = 8.5 µM</td>
<td>40,77</td>
</tr>
<tr>
<td>NQO2</td>
<td>Chem Prot, Xray, Enz Inhib</td>
<td>Binding, Kd = 35 nM</td>
<td>44,45</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Chem Prot</td>
<td>Binding</td>
<td>48</td>
</tr>
<tr>
<td>AKT-1</td>
<td>Chem Prot</td>
<td>Binding</td>
<td>59</td>
</tr>
<tr>
<td>HDACs</td>
<td>Enz Inhib</td>
<td>pan-HDACi</td>
<td>25</td>
</tr>
<tr>
<td>PDE1, 3, and 4</td>
<td>Fluor</td>
<td>IC50 = 6, 10, and 14 µM</td>
<td>8</td>
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<tr>
<td>ATM</td>
<td>Kinase assay</td>
<td>Activation(^a)</td>
<td>62</td>
</tr>
<tr>
<td>SIRT1</td>
<td>Fluor</td>
<td>Activation(^b)</td>
<td>75,76</td>
</tr>
<tr>
<td>PKGα, βI, and PKD1</td>
<td>Kinase assay</td>
<td>IC50 = 2, 100, and 800 µM</td>
<td>65,66,69</td>
</tr>
<tr>
<td>aromatase</td>
<td>Enz Inhib, (\text{in silico})</td>
<td>IC50 = 12.8 µM</td>
<td>47</td>
</tr>
<tr>
<td>DNA/RNA</td>
<td>Spec</td>
<td>Destabilizing</td>
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<td>Lipoproteins</td>
<td>HPLC</td>
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<tr>
<td>DNA polymerase α and δ</td>
<td>Enz Inhib</td>
<td>(K_i = 3.3) and (5.0) µM</td>
<td>63</td>
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<tr>
<td>F1-ATPase</td>
<td>Xray</td>
<td>Binding</td>
<td>80</td>
</tr>
<tr>
<td>CBR1</td>
<td>Chem Prot, Enz Inhib</td>
<td>Binding, (K_i = 55.8) µM</td>
<td>43</td>
</tr>
<tr>
<td>LTA4H</td>
<td>Chem Prot, (\text{in silico})</td>
<td>Binding</td>
<td>35</td>
</tr>
<tr>
<td>PPARγ and δ</td>
<td>Xray, Affin Chrom</td>
<td>(K_d = 1.4) and (2.7) µM</td>
<td>51</td>
</tr>
<tr>
<td>Various kinases</td>
<td>Activity assay</td>
<td>Moderate to no effect</td>
<td>72</td>
</tr>
<tr>
<td>TyrRS</td>
<td>Enz Inhib, Xray(^c)</td>
<td>(K_i = 22) µM</td>
<td>70</td>
</tr>
</tbody>
</table>

\(^a\)Under oxidizing conditions (\(H_2O_2\)).
\(^b\)Direct binding disproved.
\(^c\)Cocrystal with \(cis\)-resveratrol.

Enz Inhib, enzyme inhibition assay; Chem Prot, chemical proteomics; Xray, X-ray cocrystal structure; Fluor, fluorescence assay; Spec, spectroscopic assay; Affin Chrom, affinity chromatography.

Pharmaceutical, the COX inhibitor aspirin, can prevent colorectal cancer in humans.\(^31\)

COX enzymes catalyze the formation of prostaglandin H\(_2\) (PGH\(_2\)) from arachidonic acid, which is the key precursor to other prostaglandins (PGE\(_2\), PGD\(_2\), PGF\(_{2\alpha}\)), thromboxaneA\(_2\) (TXA\(_2\)), and prostacyclins (PGI\(_2\)); these prostanooids have many hormone-like effects in cells and act as autocrine and paracrine factors. COX-2 is the inducible isofrom of the COX enzyme, thought to be responsible for the inflammatory response. There have been several very successful selective COX-2 inhibitors that act as potent nonsteroidal anti-inflammatory drugs (NSAIDs), with diclofenac and ibuprofen being the archetypal examples. Resveratrol inhibits the enzymatic activity of COX-1 and COX-2 with IC\(_{50} = 0.54\) and 1.0 µM, respectively,\(^28\) as evidenced by enzyme-linked immunosorbent assay (ELISA) quantitation of PGE\(_2\) production from arachidonic acid using recombinant protein. Resveratrol was also shown to bind COX-2 via a chemical proteomics approach using both whole-cell lysate and recombinant enzyme; this binding was absolutely required for its inhibitory effects on the ability of human colon adenocarcinoma cells to form colonies in soft agar.\(^29\)

Resveratrol-mediated COX-2 inhibition reduces the production of PGE\(_2\) and inhibits the proliferation of wild-type mouse embryonic fibroblasts (MEFs) but not COX-2–deficient cells (Ptgs2\(^{-/-}\) MEFs).\(^29\)

COX-2 has been suggested as a target in the field of cancer treatment\(^32\) and prevention, but there are indications that selective COX-2 inhibitors increase the risk of cardiovascular complications such as heart attack and stroke.\(^30\)

Conversely, resveratrol has been implicated in reduced cardiovascular risk; therefore, the anticancer properties of resveratrol are probably not completely attributable to COX-2 inhibition. Indeed, aspirin has been shown to be an irreversible inhibitor of COX-2 and has also shown very promising cancer-preventive properties, in addition to its utility in protecting against heart...
attacks and strokes via antiplatelet activity. A cocrystal structure of resveratrol and COX-1 has been solved,\textsuperscript{33} showing resveratrol bound at the same site as NSAIDs like ibuprofen; indeed, the OH of Ser530 that is acetylated by aspirin forms a hydrogen bond with the 3-OH of resveratrol.

Leukotriene A4 hydrolase (LTA\textsubscript{4}H) is an epoxide hydrolase that catalyzes the final step toward the synthesis of proinflammatory leukotriene B4 (LTB\textsubscript{4}), which is a neutrophil chemoattractant. LTA\textsubscript{4}H has been highlighted as a potential target for anti-inflammatory drugs, with emphasis on chronic inflammatory conditions such as asthma and arthritis.\textsuperscript{34} LTA\textsubscript{4}H inhibition reduces pancreatic cancer cell proliferation, and resveratrol has been shown to bind to LTA\textsubscript{4}H using chemical proteomics from pancreatic cancer cell lysate and recombinant enzyme, and also to reduce proliferation in an LTA\textsubscript{4}H-dependent manner, as demonstrated by shRNA-mediated knockdown.\textsuperscript{35} LTA\textsubscript{4}H has also been shown to degrade the neutrophil chemoattractant peptide PGP,\textsuperscript{36} suggesting that selective inhibition of LTA\textsubscript{4}H may not reduce neutrophil levels and hence not reduce inflammation.

**Metabolism regulators**

Fatty acid synthase (FAS) catalyzes the synthesis of palmitate from acetyl and malonyl groups and has been observed to be highly upregulated in several cancer types; consequently, inhibition of FAS has been proposed as a potential target for therapy\textsuperscript{37,38} or adjuvant therapy, since inhibiting or reducing FAS restores chemosensitivity to drug-resistant models of breast cancer.\textsuperscript{37} Inhibition of FAS by the synthetic chemical inhibitor C75 reduces proliferation of a number of human cancer cell lines and exhibits anticancer properties in xenograft models of mesothelioma and breast, prostate, and ovarian cancer.\textsuperscript{39} Depending on the experimental design and model used, research has shown direct inhibition of FAS by resveratrol at IC\textsubscript{50} = 8.5 \mu M,\textsuperscript{40} as measured by a reduction in NADPH levels using an *in vitro* activity assay, as well as significant downregulation of mRNA and protein levels at much higher concentrations (\textasciitilde 100 \mu M), as measured by quantitative reverse transcriptase polymerase chain reaction and western blot in resveratrol-treated breast cancer cells. These data might point toward different mechanisms of action being engaged at different concentrations, although clinically achievable concentrations would favor the direct inhibitory mechanism.

Carbonyl reductase [NADPH] 1 (CBR1) catalyzes the reduction of a number of carbonyl compounds, such as prostaglandins, quinones, and menadione. CBR1 was identified as a direct target of resveratrol using a combination of chemical proteomics and an *in vitro* enzyme activity assay, wherein resveratrol inhibited CBR1 conversion of doxorubicin to doxorubicinol with \( K_i = 55.8 \mu M \) (and \( \alpha K_i = 164 \mu M \); \( \alpha = 2.98 \)). Inhibition by resveratrol was shown to slow the enzymatic reduction of doxorubicin to doxorubicinol *in vitro* and hence increase the half-life of doxorubicin and increase its efficacy against breast cancer cells.\textsuperscript{43} By temporarily inhibiting metabolic enzymes using adjuvant therapy, the effective dose of cancer chemotherapy drugs may be reduced to increase efficacy and potentially reduce side effects.

Ribosylhydronicotinamide dehydrogenase [quinone] (NQO2) is a phase II detoxifying enzyme that catalyzes the 2- and 4-electron reduction of
quinone substrates to hydroquinones using dihydronicotinamide riboside (NRH) as the electron donor. NQO2 inhibition by resveratrol has been shown to reduce leukemia and prostate cancer cell proliferation.\textsuperscript{14,45} A cocrystal structure of NQO2 with resveratrol has been solved that illustrates competitive binding at the enzymatic site,\textsuperscript{45} in close proximity to the cofactor flavin adenine dinucleotide (FAD). FAD presents a hydrophobic surface capable of forming π–stacking interactions with resveratrol, and the 3- and 5-phenol groups form hydrogen bonds with the amide nitrogen of Asn161 and the backbone carbonyl of Gly174. The 4′-OH of resveratrol is also bound via a water molecule hydrogen-bonding network to the backbone carbonyls of Asp117 and Thr71. A \( K_d \) value of 35 nM has been reported, as measured by fluorescence quenching, and an \textit{in vitro} enzymatic activity assay revealed a \( K_i \) of 50 nM. The inhibition of NQO2 may upregulate the expression of cellular antioxidant enzymes and increase cellular resistance to oxidative stress. It may even be an attractive target for Parkinson’s research, as polymorphism in the NQO2 promoter region, designated \textit{variant D}, is elevated in patient blood samples, which leads to increased expression of NQO2,\textsuperscript{46} and overexpression of NQO2 in neuroblastoma SH-SY5Y cells was associated with increased production of reactive oxygen species when exposed to exogenous dopamine.

Aromatase is a cytochrome P450 enzyme that converts C19 androgens to C18 estrogens, for example, androstenedione and testosterone to the aromatic estrogenic steroids estrone and estradiol, respectively. Inhibition of aromatase represents an effective treatment for hormone-sensitive breast cancer in postmenopausal women. Resveratrol inhibits aromatase with an \( IC_{50} \) of 12.8 \( \mu \text{M} \),\textsuperscript{47} demonstrated by an enzymatic assay using a human placental microsome source of active aromatase. Resveratrol, along with other phytochemicals, has been docked \textit{in silico} into the active site of a homology model of aromatase with good experimental agreement.

Glutathione S-transferase–π (GSTP1) catalyzes the conjugation of xenobiotics with reduced glutathione, thereby acting as a detoxifying enzyme against possible procarcinogens. GSTP1 was identified as a direct binding partner of resveratrol using a mass spectrometry/chemical proteomics approach, but the nature and potency of the interaction was not measured.\textsuperscript{48} In the same published data, estrogen receptor β (ERβ) was identified as a potential resveratrol-binding protein, but with less confidence.

**Cell signaling**

Peroxisome proliferator–activated receptors (PPARs) are members of a family of nuclear receptor proteins that act as transcription factors and comprise three isoforms; PPAR\( \gamma \), \( \delta \), and \( \alpha \). PPAR\( \gamma \) has been implicated in lung cancer,\textsuperscript{49} with PPAR\( \gamma \) agonists reducing cell proliferation in a number of cell lines, including non-small cell lung carcinoma. Resveratrol has been shown to protect against the deleterious effects of a high-calorie diet in preclinical models;\textsuperscript{50} therefore, direct inhibition of PPAR\( \gamma \) could be a viable mechanism of action. Resveratrol binds to PPAR\( \gamma \) with \( K_d \) of 1.4 \( \mu \text{M} \), measured by frontal displacement chromatography using immobilized ligand-binding domains with resveratrol and its phase II metabolites (\( K_d \) of 1.0 and 0.8 \( \mu \text{M} \) for resveratrol-3-O-glucuronide and resveratrol-4′-O-glucuronide, respectively).\textsuperscript{51} Furthermore, resveratrol displaces the antidiabetic drug rosiglitazone from the binding site with \( IC_{50} \) of 27 \( \mu \text{M} \), calculated using a luciferase reporter assay in HepG2 cells, and the X-ray structure of the cocrystal of resveratrol with the ligand-binding domain of PPAR\( \gamma \) shows resveratrol bound in a position close to the binding site of known PPAR\( \gamma \) ligands.\textsuperscript{51} Resveratrol forms hydrogen bonds between 3-OH and the backbone nitrogen of Ser342, as well as through water molecules associated with Ser342 and Arg288, and the 4′-OH forms hydrogen bonds with the backbone nitrogen of Arg280. Resveratrol also binds PPAR\( \alpha \) with a \( K_d \) of 2.7 \( \mu \text{M} \) and displaces Wy-14643 (a known PPAR\( \alpha \) agonist) with \( IC_{50} \) of 32 \( \mu \text{M} \). The authors of this work\textsuperscript{51} concluded that resveratrol acts as an antagonist of PPAR via direct binding of PPAR\( \gamma \) and PPAR\( \alpha \).

Phosphodiesterases (PDEs) are a group of enzymes that catalyze the hydrolysis of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to AMP and GMP, respectively. As such, they play key roles in the cAMP-dependent pathway of intracellular signal transduction; therefore, inhibition or activation of PDE enzymes can influence the concentration of this secondary messenger. PDE inhibitors are used to treat a range of conditions, and PDE inhibition
by resveratrol has been shown to increase cAMP levels and increase SIRT1 activity via the CamKKβ–AMPK pathway. PDE1 inhibitors, such as vincristine, may be useful in the treatment of cognitive decline and Alzheimer’s disease; PDE3 inhibitors like milrinone are used to increase cardiac output in heart failure; and the PDE4 inhibitor roflumilast is used in the treatment of COPD to reduce inflammation. Resveratrol has been shown to inhibit PDE1, 3, and 4 with IC_{50} = 6, 10, and 14 μM, respectively, which was determined using a tritiated cAMP substrate and recombinant enzymes. PDEs are clearly a group of protein targets that require more consideration with respect to resveratrol’s biological effects.

**Cell cycle regulation**

One of the key characteristics of malignant cells is dysregulation of the cell cycle, leading to evasion of apoptosis, increased proliferation, and the accumulation of multiple oncogenic mutations. Selective programmed cell death of diseased or prediseased cells has been postulated as a potential mechanism of action, especially in the field of cancer treatment and cancer chemoprevention. Induction of apoptosis by resveratrol via Bcl-2 family mechanisms, p53 activation, and CDK regulation has been reported, as well as resveratrol causing autophagy at clinically achievable concentrations.

RAC-α serine/threonine protein kinase (AKT1) is one of three closely related kinases that mediate processes including metabolism, proliferation, angiogenesis, and cell survival via phosphorylation of substrate proteins such as FOXO, BAD, GSK-3α/β, and Casp9. Direct binding of resveratrol was shown using a chemical proteomics approach, and the interaction of resveratrol with AKT1 may work in parallel with NQO2 inhibition to control cyclin D1 and modulate the proliferation of cells that express high levels of NQO2 or AKT1. The AKT pathway is of interest in cancer research because overactivation of AKT kinases is common in many human cancers and results in tumor cell survival and increased resistance to apoptosis; therefore, direct inhibition of AKT-1 by resveratrol presents an attractive mechanism of cancer prevention.

Serine protein kinase ataxia telangiectasia mutated (ATM) activates checkpoint signaling upon genotoxic stress, especially DNA double-strand breaks, to trigger cellular repair mechanisms, and the loss of ATM activity has been observed in various tumor types. Resveratrol binds to ATM to activate it and increase autophosphorylation and substrate phosphorylation, as demonstrated by H2O2-induced phosphorylation of p53 using purified ATM enzyme. This activation of ATM under genotoxic insult may afford protection in an antioxidant-type mechanism.

Calf thymus DNA polymerases (Pol) α and δ are inhibited by resveratrol with Ki = 3 μM and 5 μM, respectively (determined by measuring radio-label incorporation into DNA in an enzyme activity assay), which may contribute to resveratrol’s antiproliferative action. Resveratrol inhibited both Polα and δ to induce cell cycle arrest at S phase in normal fibroblasts and HT1080 fibrosarcoma cells. DNA replication dysfunction is inexorably linked with the carcinogenic process; therefore, certain DNA polymerases that help cancer cells tolerate DNA damage have been proposed as potential targets for therapies.

Protein kinases C (PKC) are a large family of serine/threonine kinases that consist of 15 isoforms that can be divided into three classes depending on their requirements for activation: conventional, needing diglyceride (DAG), Ca^{2+}, and phospholipid; novel, requiring DAG but not Ca^{2+}; and atypical, requiring neither Ca^{2+} nor DAG. PKCα and βI have been reported to be inhibited by resveratrol with IC_{50} = 2 and 100 μM, respectively, as measured by the rate of phosphorylation of a peptide substrate. PKCs are considered to be attractive targets owing to isoforms being activated in several disease states including cancer, diabetes, ischemic heart disease, heart failure, Parkinson’s disease, and Alzheimer’s disease. PKCα (conventional) has been implicated in proliferation and metastasis in cancer and in heart failure and PKCβI (conventional) has been implicated in cancer cell invasion and diabetic complications. Although there are no blockbuster PKC inhibitors to date, it is clear that this class of kinase offers an interesting mechanism of action against several of the diseases that resveratrol has been shown to have activity against.

Serine/threonine protein kinase D1 (PKD1) is also a member of the PKC family of proteins that helps regulate multiple signaling pathways. Dysregulation of PKD1 expression is implicated in various cancer phenotypes, including cell proliferation, apoptosis, adhesion, and motility. Resveratrol has...
been shown to inhibit PKD1 with an IC$_{50}$ of 200 μM, which was measured by immunoblot quantitation of autophosphorylated protein in COS-7 cells. It remains unclear whether it is desirable to inhibit or activate PKD1 with respect to cancer therapy, and it might actually be tissue/cancer type dependent.

Tyrosyl transfer RNA synthetase (TyrRS) is a class I amino acid tRNA synthetase that catalyzes the specific attachment of tyrosine to its respective tRNA. Under stress conditions such as serum starvation in vitro, TyrRS translocates to the nucleus and may activate poly (ADP-ribose) polymerase 1 (PARP1). Resveratrol has been shown to bind and inhibit TyrRS with an inhibition constant of $K_i = 22$ μM in an ATP–pyrophosphate exchange assay. Resveratrol was also shown to activate the same pathways as serum starvation in HeLa cells and to show increased autopharylation of PARP1, as well as increased phosphorylation of AMPK in a time-dependent manner. A cocrystal structure of mini-TyrRS (catalytic subunit) with cis-resveratrol was also solved, with the authors suggesting that TyrRS promotes double-bond isomerization in resveratrol, which in turn promotes a conformational change in TyrRS. The cocrystal structure shows cis-resveratrol forming hydrogen bonds at the 4′-OH with Tyr39 and Asp173 and at the 3-OH with the carbonyl of Gln170.

**Posttranslational modification**

Posttranslational modifications (PTMs), including phosphorylation, acetylation, oxidation, methylation, glycosylation, and ubiquitination, are key to controlling cellular function. These modifications of the protein structure act as molecular switches and can have profound effects on the structure, function, activity, and localization of the target protein. Kinases have been studied extensively, as they control a vast number of cellular pathways via phosphorylation, and HDACs have become increasingly investigated owing to their regulation of histone structure and subsequent control of DNA replication via deacetylation.

Through medium- and high-throughput screening, resveratrol has been shown to inhibit a number of kinases and HDACs. However, typically, these screens are used for ligand hit identification with an emphasis on finding nanomolar affinity compounds to develop into drug or tool compounds, meaning that many low-affinity interactions may go unreported or be considered a consequence of resveratrol being a pan-assay–interfering compound (PAINS). A number of kinases were shown to be moderately inhibited by resveratrol in a Medical Research Council (MRC) 33P-ATP filter-binding assay screen. PKCs, AKT1, and PKD1 (discussed above) were demonstrated to be inhibited by resveratrol with 58%, 63%, and 51% activity remaining, respectively, at 50 μM.

Resveratrol has been described as a pan-HDAC inhibitor and has been found to have moderate inhibitory activity against HDACs 1–11, using a fluorometric assay kit and the individual isolated enzymes, with several HDACs showing 30% inhibition at 50 μM. The HDAC family enzymes are currently considered promising targets with respect to cancer treatment and, encouragingly, HDAC inhibitors appear to act selectively on cancer cells. It is unclear whether moderate inhibition by resveratrol at relatively high concentrations would have significant biochemical implications, but it is nonetheless an interesting direct binding partner.

The NAD-dependent protein deacetylase sirtuin-1 (SIRT1) is a deacetylase with a broad range of functions. When SIRT1 is knocked out in mouse models, it causes metabolic derangements, infertility, and impairment of normal cognitive function, whereas overexpression increases life span, improves insulin sensitivity, and reduces other harmful effects of obesity in mice. In fact, activation of SIRT1 has been postulated as a mechanism for a number of beneficial effects, including reduced cardiovascular disease, frailty, osteoporosis, inflammation, stress susceptibility, diabetes, cancer, and neurodegeneration.

Activation of SIRT1 has been shown to mimic dietary restriction, the benefits of which have been demonstrated in vivo, with increased life expectancy and delayed onset of age-related diseases, although human trials are undeniably problematic. The activation of SIRT1 by resveratrol has been a contentious issue for several years; it was initially shown to result from direct binding of resveratrol to the protein, but subsequent studies have demonstrated this mechanistic observation was caused by an experimental false positive. However, there now seems to be consensus that resveratrol activates SIRT1, but the mechanisms are currently unresolved. Indirect activation is regarded as a plausible mechanism of action for resveratrol with respect to...
age-related chronic disease, owing to the plethora of pathways SIRT1 acts upon.

Conclusions

Owing to the planar stilbene motif of resveratrol, it is relatively hydrophobic and hence has a greater affinity for hydrophobic pockets and binding sites in proteins. Increased affinity and specificity of binding arises from the phenolic groups at the 3, 4′, and 5 positions, where these polar OH groups act as both hydrogen-bond donors and acceptors, which can form multiple interactions with amino acid side chains as well as backbone amide groups. This is evidenced where cocystal structures have been solved.

Despite the large body of research relating to resveratrol as a compound that is potentially beneficial to human health, the mechanisms of action have yet to be fully crystallized. This is of paramount importance because current research suggests the major indications for resveratrol use are, in general, for disease prevention and/or the management of chronic conditions; therefore, robust mechanisms are required to ensure that the most relevant biomarkers of efficacy are identified and can be reliably transferred to the clinic. With advances in the field of chemical proteomics using mass spectrometry and the increasing use of nuclear magnetic resonance to study interactions, there will undoubtedly be more supposition as to the major biological targets of resveratrol. There is little doubt that resveratrol is biologically active and will exert its effects via a number of mechanisms, but these mechanisms may alter depending on cell type, dose, and dosing schedule, as well as any coadministered pharmaceuticals.

Conflicts of interest

The authors declare no conflicts of interest.

References


