Resveratrol: Challenges in Translation to the Clinic — A Critical Discussion

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Abstract

Low cancer survival rates and the serious side effects often associated with current chemotherapeutics highlight the need for new and effective nontoxic anticancer agents. Since 1997 when Jang and colleagues first described resveratrol’s ability to inhibit carcinogenesis, it has consistently proven effective at tumor inhibition in diverse human cancer models. This finding has raised the hope that resveratrol would pioneer a novel class of nontoxic chemotherapeutics. As a consequence of initial basic and preclinical studies, resveratrol is now being extensively promoted in the unregulated nutraceutical sector. However, some fundamental aspects of resveratrol’s action need to be understood before it can be developed into a clinically viable anticancer drug. These areas pertain to the key mechanism(s) by which resveratrol potentiates its antitumor effects. Current research suggests that these mechanisms might be through novel pathways, requiring an understanding of cellular uptake, sentinel targets, and in vivo biological networks. The metabolism of resveratrol and its bioavailability also warrant further consideration in light of recent in vitro and in vivo studies. Finally, we need to appreciate the sorts of information about resveratrol that may translate between different disease entities. We present a critical discussion of these issues and suggest important experiments that could pave the way to the successful translation of resveratrol to the clinic.

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Now that 60 Minutes, Barbara Walters, National Public Radio, Dr. Oz, and a myriad of columnists at the New York Times and other newspapers have offered their comments and recommendations about the health benefits of resveratrol, including its potential anticancer properties, perhaps it is time for scientists to ask a few essential questions about the popular product before considering a further pitch to the public.

In 1997, Jang and colleagues published their seminal report on resveratrol’s ability to inhibit the three major stages of carcinogenesis (1). Since then, there has been an explosion of literature about resveratrol’s effects on cancer. Some fundamental aspects of our understanding of resveratrol, however, are yet to be resolved. These gaps in our knowledge must be addressed before the full potential of this nontoxic compound as an anticancer drug can be realized.

Cellular Uptake

What evidence do we have that resveratrol actually enters cancer cells?

A critical first step to delineate the mechanisms of drug action is to determine whether resveratrol mediates its effects by cell surface receptors or intracellular targets in tumor and other cells. Both intra- and extracellular resveratrol targets have been proposed (see below, “Targets: Fast and Slow”). However, a direct-binding partner has yet to be convincingly established. Elucidation of whether resveratrol enters cells or acts at the cell surface will shed some light on the targets on which it acts.

Resveratrol is a hydrophobic compound and, currently, the only compelling evidence for entry of resveratrol into cells comes from highly specialized cell types: intestinal and liver cells. When a monolayer of colonic Caco-2 cells was treated in vitro with resveratrol, a saturating dose-dependent apical to basolateral transport was observed (2, 3). In another study, the human hepatoblastoma cell line HepG2 and human hepatocytes were used to compare resveratrol transport in normal and tumor cells (4). Resveratrol uptake was detected by its intrinsic fluorescence properties as well as by using radiolabeled drug. Concentration and temperature dependence of uptake were examined, and results indicated that both passive diffusion and carrier-mediated transport might be involved.

The above cell types, however, have specialized uptake mechanisms. The intestinal epithelium is adapted for...
absorption of nutrients from food. A study of drug transport using a Caco-2 monolayer may indicate transepithelial transport of a drug, but does not necessarily imply that other cells have similar uptake mechanisms. Similarly, one of the main functions of hepatocytes is to extract protein-bound drugs and metabolites from circulation. Consequently, hepatocytes are equipped with many different transport proteins in the basolateral membrane. The only other evidence of possible resveratrol uptake has been shown indirectly through sulfotransferase 1A1 activity associated with two breast cancer cell lines, resulting in the conversion of exogenously added resveratrol to resveratrol 3-O-sulfate (5). These studies have not been replicated in other tumor cells.

If resveratrol cannot be shown to efficiently enter all tumor cells, it may be more likely that its antitumor effect is the result of binding to an extracellular target. Therefore, further studies are necessary to directly address cellular uptake in cancer cells. New technology, such as two-photon microscopy, may be particularly appropriate for detecting the real-time uptake of unmodified resveratrol and its metabolites by taking advantage of their intrinsic fluorescence under conditions of minimal photo-bleaching. Results of such studies will indicate the validity of intracellular targets identified thus far and aid in the identification of new sentinel targets.

**Targets: Fast and Slow**

What are the direct targets of resveratrol, and can we differentiate binding events from indirect effects of resveratrol?

The wealth of resveratrol literature is, in part, a result of the myriad pathways and molecules that are altered in response to resveratrol treatment (reviewed in ref. 6). Some of these effects may be cell-type specific, tissue-type specific, or dependent on whether a cell is transformed. Through parametric analysis of gene set enrichment (PAGE analysis), resveratrol has been found to cause a significant alteration in 127 pathways (7). Certain effects of drug treatment are measurable in a matter of seconds (8), whereas others only become apparent after hours or even days. This finding, then, brings up the question of which of these effects is a direct result of resveratrol binding and which are downstream effects of the initial target interaction.

Several molecular targets have been postulated as mediators of resveratrol’s anticancer effects (7). Approaches such as a phage-display screen using biotinylated resveratrol identified several candidates (9). However, the direct binding of resveratrol to these targets in a cellular context has yet to be convincingly shown.

An example of a direct-binding target identified through column chromatography is quinone reductase 2 (10). The binding constant for NQO2 was in a physiologically relevant range. However, not all tumor cells have measurable levels of this protein, despite having similar sensitivity to the drug (11). Therefore, binding to this protein may not be part of a general mechanism for resveratrol antitumor action.

Other targets reported include DNA polymerase α, PKC, PKD, COX2, ribonucleotide reductase, and F0/F1 ATPase/ATP synthase, on the basis of alterations of enzymatic activity. Only F0/F1 ATPase/ATP synthase, however, has been shown to interact directly with resveratrol. An additional complication in identifying and validating such targets is that binding and enzymatic experiments in vitro or in cells in culture may not reflect in vivo conditions. Because levels of unmetabolized resveratrol in vivo do not exceed the low micromolar range (11), it is possible that some observed target binding and modulations of pathways are only induced because of exposure of isolated enzymes or cells to constant and high drug concentrations in vivo.

A more recent group of potential targets, the sirtuins, has been the subject of intense research as mediators of resveratrol's life-extending activity as well as antitumor activity. The direct binding of resveratrol to sirtuins has not been shown. Early work showing direct resveratrol activation of purified SIRT1 using a fluorescently modified substrate for sirtuin was shown to be an artifact of the assay (12). Additionally, the effects of resveratrol on sirtuins in cells seem to be measurable only after several hours. This finding is suggestive of downstream or indirect effects, if any at all. More recent studies also conflict with the original observation that resveratrol has an effect on Sir2 activity in vivo (13), or an effect on the lifespan of yeast (13), *Drosophila*, or *Caenorhabditis elegans* (14).

Extracellular receptors for resveratrol such as insulin growth factor receptor (IGFR) and integrin αvβ3 have been proposed. The IGFR study compared downstream activity using known ligands (15). No direct binding was shown in this study. The αvβ3 study relied on competition assays using a known ligand and binding studies using membrane fractions (16). Radiolabeled resveratrol was used to suggest
direct binding to the β3 subunit of the integrin. However, given its properties, resveratrol is likely to associate with membranes readily, making it difficult to assess the specificity of these binding studies. Even though further work is needed to establish that resveratrol binds to these extracellular receptors, these studies suggest that resveratrol could activate several extracellular targets.

One of the very early in vitro events observed in tumor cells following resveratrol addition is an increase in intracellular calcium, which can be measured within seconds after drug treatment (8). Calcium signaling (Fig. 1) may be a shared early response underlying the nontoxic feature of different anticancer natural products (17). If so, it may be possible to define a novel calcium-based mechanistic model for the development of a new generation of nontoxic chemotherapeutics using resveratrol as a model compound. However, the sentinel targets involved in calcium activation are yet to be identified. Further, we have to recognize that some binding targets for resveratrol activate early events, whereas others might potentiate later events.

Despite important progress, the binding targets of resveratrol that mediate its anticancer activity remain unknown. The relevant targets mediating effects in vitro versus in vivo might differ, and there seem to be several targets within a single cell type as well as differences between cell types. To better understand the mechanisms through which resveratrol mediates its anticancer properties, studies need to better reflect conditions in vivo at drug levels that lead to inhibition of tumor growth.

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Fig. 1. Resveratrol-induced $\text{Ca}^{2+}$-mediated apoptosis. Resveratrol activates the inositol triphosphate receptor (IP3R) either through an extracellular receptor or through an intracellular target resulting in release of extracellular receptor calcium, reflected in a rapid increase in intracellular calcium ($[\text{Ca}^{2+}]_i$). The subsequent calcium uptake by the mitochondria causes mitochondrial membrane depolarization ($\Psi_m$), increase in reactive oxygen species production (ROS), release of cytochrome c and smac/diablo, activating the intrinsic apoptotic pathway via caspases-9 and -3. Mitochondrial calcium-induced-calcium-release (mCICR) results in the activation of calpain, which then cleaves various substrates including calcium exchangers and pumps, like PMCA1, leading to additional elevation of intracellular calcium, further stimulating cell death.
**Resveratrol Actions**

**Is resveratrol antiproliferative, proapoptotic, and antiangiogenic?**

*In vitro* studies of resveratrol using a variety of tumor cell lines have shown the drug to be antiproliferative as well as proapoptotic, through the activation of many different pathways. Resveratrol inhibits cell-cycle progression by modulating major cell-cycle regulators, arresting cells at the G1/S, S, or G2/M phase, and by inhibiting DNA synthesis. These effects, however, have not been shown *in vivo*. The mechanisms causing tumor inhibition, therefore, are unclear, even though tumor inhibition is observed in many different animal models.

Alternatively, it is well established that angiogenesis is critical to tumor progression. Resveratrol has been reported to have an antiangiogenic effect in certain tumor models, as indicated by decreased vessel density in treated tumors (18–21). However, an observed decrease in microvessel density cannot differentiate between cause and effect. Either the inhibition of tumor growth results in fewer blood vessels or an antiangiogenic effect causes decreased tumor outgrowth because of a lack of nutrients and oxygen. The tumor-inhibitory effects *in vivo* could be due either to direct effects of resveratrol on tumor cell growth or indirect effects on angiogenesis. An initial study in a mouse model of Lewis lung carcinoma indicated a resveratrol effect on neovascularization (19). However, more *in vivo* studies are needed to show a direct effect of resveratrol on blood vessel formation as the mechanism for tumor inhibition using cell-specific reporter systems.

In contrast to tumor inhibition, higher local doses of resveratrol result in massive tumor cell death and tumor regression as indicated by terminal deoxynucleotidyl transferasemediated dUTP nick end labeling (TUNEL) staining and histology (11). Thus, the reported proapoptotic effects of resveratrol may only be relevant when local concentrations of resveratrol more closely match the higher concentrations studied *in vitro*. Therefore, strategies to improve the bioavailability of resveratrol either systemically or locally will provide more potent anticancer effects.

**Bioavailability**

**How do we reconcile the anticancer effects of resveratrol determined from *in vitro* studies with *in vivo* measurements of resveratrol bioavailability?**

Preclinical studies using resveratrol in animal models of different human cancers indicate that resveratrol has potent antitumor properties. However, measurements of serum levels of unmetabolized resveratrol suggest that its bioavailability is very low after oral or intraperitoneal administration of the drug. This low bioavailability is mainly due to poor absorption and ready metabolism to glucuronidated and sulfated compounds, followed by rapid clearance of these metabolites. Serum levels of unmetabolized resveratrol peak in the sub- to low-micro- molar range (<3 μmol/L) within minutes of oral drug administration, and decrease rapidly thereafter (11, 23–26). One implication of these findings is that the direct activation of cellular targets by resveratrol needs to reflect this transient occurrence of both resveratrol and the metabolites. In addition, no accumulation of resveratrol in tumors or normal tissues has been found after extended treatment regimens (11). These data contrast sharply with *in vitro* studies wherein sustained concentrations of tens of micromolars are required to obtain substantive antitumor effects. This apparent conundrum was discussed elegantly by Gescher and Steward (27). These observations may suggest one of two different things: (a) the mechanisms of action of resveratrol are different *in vivo* and *in vitro*; or (b) the *in vitro* conditions for growing cells somehow alter the sensitivity to resveratrol. In the first case, the antiangiogenic activity of resveratrol could indirectly inhibit tumor growth. Such properties of the tumor environment are not recapitulated *in vitro*. In the second case, growth of tumor cells on a two-dimensional surface, the presence of high concentrations of growth factors, high O2 levels, and lack of inhibitory cell-cell contacts with surrounding normal cells could decrease the sensitivity of cells to resveratrol. These optimized growth conditions for tumor cells in culture may necessitate higher drug concentrations *in vitro*.

Reports of the relevant serum levels of resveratrol are further complicated by recent studies and unpublished observations indicating that resveratrol metabolites do not inhibit tumor cell growth (refs. 28, 29; and L. Subramanian, unpublished observations). This finding suggests that glucuronidation and sulfation not only target the drug for removal from the body, but also mitigate its antitumor activity. Minor conversion back to resveratrol by local sulfatases, for example, would not compensate for this rapid decrease in activity. To increase the potency of resveratrol treatment, it will be necessary to increase the amount of unmetabolized compound at the tumor site. A major obstacle to attaining this goal systemically is the low solubility of the drug. Future research should be directed toward new formulations and analogues that increase its bioavailability and delay its metabolism (Fig. 2).

The bioavailability conundrum also brings up the issue of resveratrol’s ability to differentiate between normal cells and cancerous cells in animal models. At low concentrations in which there is no evidence of cell death either in normal or tumor tissue, there is, nevertheless, an antitumor effect of the drug leading to tumor inhibition. At higher local concentrations in which there is significant evidence for cell death in tumor tissue, the surrounding normal tissue remains unaffected (11). This discrimination between normal and tumor cells could arise due to differences in the cellular uptake of resveratrol, the expression of cellular targets, or a differential ability to modify resveratrol into less active forms. Studies of these processes are necessary to understand the non-toxic nature of resveratrol.
Consistencies between Disease Processes

Can the findings from resveratrol research done in the realm of cancer be generalized to cardioprotection, life prolongation, diabetes prevention, and vice versa?

Resveratrol has a wide variety of pharmacologic activities, making its study relevant to several diseases such as cancer, cardiovascular and neurodegenerative diseases, and diabetes. However, it is not always clear how the same drug causes different or even opposite effects in different cell types and in different diseases. For example, resveratrol causes cell death in tumor cells but is effective as an antiapoptotic or protective agent in nerve cells (30). Similarly, resveratrol is antiangiogenic in certain tumor models yet proangiogenic in the case of...
myocardial infarction (31, 32). Resveratrol increases mitochondrial health in muscle cells, whereas it induces mitochondria-mediated apoptosis in tumor cells (33). How might these effects occur? Although most direct targets of resveratrol may be expressed in a variety of different cell types, albeit at different levels, intracellular signaling networks will be different depending on the cell type and cell status (nondividing, dividing, tumorigenic), leading to different outcomes following initial target interaction.

Epidemiologic studies suggest a correlation between diabetes and cancer risk. Resveratrol has been shown to exhibit antidiabetic effects. A recent report of the use of the antidiabetic drug metformin in cancer treatment suggests that resveratrol’s antidiabetic effects may be relevant to its antitumor effects as well (32, 34). Therefore, findings of resveratrol action will be of potential cross-disease importance. As different molecular pathways are elucidated in greater detail and are incorporated into larger networks, it will become increasingly apparent how the addition of a drug like resveratrol can still be effective in very disparate diseases.

Many of these diseases are prevalent simultaneously, particularly in elderly patients. On the basis of current literature, it would seem that resveratrol could ameliorate secondary conditions even as it is used to treat a primary disease. However, it is necessary to carry out comprehensive clinical trials on both the preventive potential as well as curative efficacy of resveratrol in different diseases before such claims can be validated.

Market Claims

What can we tell the public?

Resveratrol has great potential as an anticancer drug either as primary or as adjuvant therapy. One of its major benefits is a lack of toxicity at doses needed for its antitumor effect. Toxicity remains one of the major concerns with current chemotherapies, and resveratrol, therefore, may allow for lowering of the doses of toxic compounds while maintaining or enhancing the antitumor efficacy. Furthermore, there have been no reports of acquired resistance to resveratrol during treatment.

At this time no clinical trials have been completed to indicate that resveratrol will be effective as an anticancer treatment in humans. All the studies to date have been done in tissue culture models or animal models. These studies show a strong antitumor effect of resveratrol with oral dosing and the potential for even higher efficacy if doses can be further increased by overcoming delivery and solubility obstacles. However, as has been shown with numerous other potential drugs, results from animal studies do not necessarily extrapolate to humans. Therefore, great caution has to be taken not to raise expectations of success in using this compound as an anticancer agent before clinical trials have been successfully conducted. Even as these trials proceed, the concerns raised in previous sections should be adequately addressed in order to maximize resveratrol’s potential as a nontoxic anticancer drug. Currently, one trial is underway in colon cancer patients, with trials for a number of other types of cancer proposed. Ultimately, based on the results of the clinical trials, the Food and Drug Administration (FDA) alone has the authority to approve the use of resveratrol in cancer therapy in the United States. In addition, its antioxidant effects, currently being tested in other clinical trials, may lead to its use as an approved chemopreventive agent or in combination with other anticancer drugs. Again, the nontoxic aspects of resveratrol also make it a likely candidate for neoadjuvant or adjuvant treatment.

Nevertheless, resveratrol is already available as a nutritional supplement. Because nutraceuticals are more loosely regulated by the FDA, much stronger claims about resveratrol’s benefits to human health and disease are touted by this industry. Because those afflicted with cancer are desperate for a cure and are distrustful of current chemotherapy, many turn to nutraceutical products, drawn by the promise of a successful “natural” treatment. Unfortunately, many of these claims about resveratrol go well beyond current research results, leading to false hopes in patients. These expectations are increased further by reports in newspapers and on television promoting resveratrol’s anticancer and anti-aging properties along with a host of other health benefits. All of these claims are yet to be scientifically proven in humans. It is not surprising that companies with a commercial interest in resveratrol may oversell its qualities in order to promote their product. However, it is important that researchers provide accurate information through peer-reviewed publications and to the public via news outlets and other avenues indicating the limitations of the studies done to date. As results of human trials become known, we will find out whether the initial promises can be fulfilled.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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