Resveratrol-induced Autophagocytosis in Ovarian Cancer Cells

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ABSTRACT

Resveratrol (3,5,4-trihydroxystilbene), a natural phytoalexin present in grapes, nuts, and red wine, has antineoplastic activities. Several molecular mechanisms have been described to underlie its effects on cells in vitro and in vivo. In the present study, the response of ovarian cancer cells to resveratrol is explored. Resveratrol inhibited growth and induced death in a panel of five human ovarian carcinoma cell lines. The response was associated with mitochondrial release of cytochrome c, formation of the apoptosome complex, and caspase activation. Surprisingly, even with these molecular features of apoptosis, analysis of resveratrol-treated cells by light and electron microscopy revealed morphology and ultrastructural changes indicative of autophagic, rather than apoptotic, death. This suggests that resveratrol can induce cell death through two distinct pathways. Consistent with resveratrol’s ability to kill cells via nonapoptotic processes, cells transfected to express high levels of the antiapoptotic proteins Bcl-xL and Bcl-2 are equally sensitive as control cells to resveratrol. Together, these findings show that resveratrol induces cell death in ovarian cancer cells through a mechanism distinct from apoptosis, therefore suggesting that it may provide leverage to treat ovarian cancer that is chemoresistant on the basis of ineffective apoptosis.

INTRODUCTION

In the United States, epithelial ovarian carcinoma is the leading cause of death among patients with gynecological cancers. It is the fifth most frequent cause of cancer death in women, and 15,000 women die of this disease annually (1). Although the majority of tumors initially respond to chemotherapy, unfortunately, most patients succumb to recurrent tumors that are composed of chemotherapy-resistant clones. Despite improved survival for many malignant gynecological conditions during the recent era in clinical oncology, the 5-year survival for patients with advanced stage ovarian cancer has remained unchanged (<20%) over the past 20 years (2).

Resistance of recurrent disease to cytotoxic drugs is the principal factor limiting long-term treatment success against ovarian cancer. The oncogenesis of ovarian cancer in particular appears to favor the development and subsequent expansion of cell clones that are resistant to apoptotic triggers. The basis for failed apoptosis and more specifically the cause of chemotherapy resistance in ovarian cancer is multifactorial. Molecular mechanisms implicated to date include expression of P-glycoproteins (e.g., multidrug resistance pumps), p53 mutations, and high-level expression of Bcl-2 and other inhibitors of apoptosis that block caspasases and stabilize the mitochondrial permeability pore (3–5). Therefore, an important research objective is the identification of lead compounds that circumvent the resistance mechanisms that limit the success of conventional drugs.

Resveratrol (3,4′-trihydroxystilbene), a natural product from grapes that is present in significant concentrations in red wine, inhibits in vitro cell growth of leukemias, prostate, breast, and other epithelial cancers (6–13). Moreover, resveratrol blocks the development of

MATERIALS AND METHODS

Chemicals. Resveratrol, monodansylcadaverine (MDC), and 3-methyladenine (3-MA) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Resveratrol was dissolved in aqueous DMSO and delivered to cells in media containing this solvent at a final concentration of 0.1% (v/v). Benzoxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (z-VAD-fmk), benzoyloxycarbonyl-Leu-Glu-Thr-Asp-fluoromethyl ketone (z-LETD-fmk), and benzoxycarbonyl-Leu-Glu-His-Asp-fluoromethyl ketone (z-LEHD-fmk) were obtained from Enzymes Systems Products (Livermore, CA).

Cell Lines, Plasmids, and Immunoblotting. Ovarian cancer cell lines A2780 and CaOV3 were obtained from the American Type Culture Collection (Manassas, VA). Dr. K. Cho (University of Michigan) generously provided ES-2, TOV112D, and A1947 cell lines. Ovarian cancer cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc., Carlsbad, CA). A2780 cells were transfected with pcDNA3-Bcl-xL, control pcDNA3 plasmid, pSFVneo-Bcl-2, or control pSFFVneo plasmid, using Lipofectamine (Life Technologies, Inc.). Individual cell clones were selected for growth in the presence of G418 (500 μg/ml; Bio-Rad Laboratories, Hercules, CA) by limiting dilution. Expression of Bcl-xL and Bcl-2 in single-cell clones was analyzed by immunoblotting. Cytosolic extracts were prepared as described (20). Apaf-1 was detected with a monoclonal antibody obtained from Trevigen (Gaithersburg, MD). Monoclonal antibody for caspase-9 was obtained from Oncogene Research.

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Stressgen (San Diego, CA). The monoclonal antibody for Bcl-xL was obtained from BD Transduction (Franklin Lakes, NJ). The monoclonal antibody for cytochrome c and polyclonal antibody for caspase-3 were obtained from BD Pharmingen (San Diego, CA). The monoclonal antibody for β-tubulin was obtained from Sigma-Aldrich. The monoclonal antibody for cytochrome oxidase subunit IV was obtained from Molecular Probes (Eugene, OR). Western blots were developed by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). Densitometry analysis was performed using Scion Image 4.02 software from Scion Corp. (Friedrick, MD).

Subcellular Fractionation. For the preparation of subcellular fractions, cells were harvested, washed with cold PBS, suspended in buffer A [20 mM HEPES-KOH (pH 7.5), 10 mM KCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 0.1 mM phenylmethylsulfonyl fluoride] and homogenized with 10 strokes with a Dounce homogenizer. Nuclei were pelleted at 1,000 × g for 10 min (4°C). The mitochondrial fraction was harvested by centrifugation at 10,000 × g for 30 min (4°C). The supernatant was harvested as the cytoplasmic fraction (S-100 extract). The purity of fractions was tested by immunoblotting with antibodies specific for either β-tubulin (cytoplasmic protein) or cytochrome c oxidase (mitochondria membrane protein).

Analysis of Viability and Apoptosis. Cells were plated, and 24 h after plating, 50–200 μM resveratrol was added to the culture medium. The percentage of apoptotic cells was determined at the indicated time points by propidium iodide (PI) staining on semi-permeabilized cells as described previously (21). In this assay, apoptotic cells are identified on the basis of hypodiploid DNA content that results from DNA fragmentation (22). Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining was performed using the Apoptosis Detection System kit (Promega, Madison, WI). To assess viability, intact cells were harvested, stained with PI, and evaluated by flow cytometry for plasma membrane permeability to PI.

Fractionation of Cytosolic Extracts by Gel Filtration. A2780 cells were treated with resveratrol (50 μM) or vehicle control (DMSO, 0.1%) for 24 h, and S-100 cytosolic extracts were prepared as described above. Two mg of S-100 extracts were loaded on a Superdex-200 HR gel filtration column (Amersham Pharmacia Biotech, Piscataway, NJ) pre-equilibrated with buffer A (see above) at a flow rate of 0.5 ml/min using a Bio-Rad BioLogic HR Workstation. The column was calibrated with Amersham Pharmacia Biotech HMW gel filtration standards (thryoglobulin, M, 669,000; ferritin, M, 440,000; catalase, M, 232,000; BSA, M, 66,000). After discarding the majority of the void volume, 400-μl fractions were collected. Aliquots (50 μl) of each fraction were resolved by SDS-PAGE and immunoblotted to detect Apaf-1 and caspase-9.

Ultrastructural Characterization. For transmission electron microscopy (TEM), treated cell monolayers were rinsed with 0.1 M Sorensen’s buffer (pH 7.4) and then fixed for 1 h in glutaraldehyde (2.5% w/v) in the same buffer. After a buffer rinse, they were postfixed for 15 min in osmium tetroxide (1% w/v), rinsed in water, and then en block stained for 30 min in aqueous uranyl acetate (3% w/v). Cell monolayers were then scraped and pelleted, dehydrated in a graded series of ethanol baths, then infiltrated and embedded in Spurr’s resin. Ultrathin sections were poststained with uranyl acetate and lead citrate and viewed on a Philips CM100 at 60 kV.

Fluorescence Microscopy. Hoechst staining was performed to evaluate nuclear morphology. Cells were incubated with Hoechst 33258 (5 μg/ml) for 15 min at ambient temperature. Wet-mounts were visualized by a combination of differential interference contrast and fluorescence microscopy (Leica DMLB, Wetzlar, Germany). Images were captured using a using a SPOT RT camera (Diagnostic Instruments Inc., Sterling Heights, MI).

Autophagic vacuoles were specifically identified using MDC. Cells, growing on glass coverslips, were treated with resveratrol or vehicle control as indicated, and after treatment, the medium was exchanged to contain 50 μM MDC in PBS and incubated for 10 min at 37°C. After incubation, coverslips were washed four times with PBS and immediately analyzed by microscopy to detect fluorescent staining by MDC (Leica DMLB). Images were captured using a SPOT RT digital camera (Diagnostic Instruments).

Statistical Analysis. When appropriate, statistical significance was tested using a two-tailed Student’s t test; P < 0.05 was considered significant. All values shown are means with the corresponding SE.

RESULTS

Resveratrol Induces Cell Death and Growth Inhibition in Ovarian Cancer Cells. Treating A2780 cells with resveratrol resulted in cell death by 24 h (Fig. 1A). Proliferation of resveratrol-treated A2780 cultures were analyzed over 72 h of continuous treatment during which time there was no further growth (Fig. 1B). To determine whether the cytotoxic response to resveratrol was unique to A2780 cells, four additional ovarian cancer cell lines were tested. Resveratrol-induced cell death in each line (Fig. 1C). Interestingly, in experiments designed to measure viability with flow cytometry, we found that resveratrol caused an unexpected, pronounced shift in the forward-side scatter parameters of A2780 and CaOV3 cells (Fig. 1D). These changes in light scatter suggested that treatment caused cells to become larger (increase in forward scatter) and more complex (increase in side scatter). This was surprising because apoptotic death is typically associated with rapid cell shrinkage (23).

Resveratrol Affects Cycle Progression and Blocks Proliferation. Although it was apparent that resveratrol was cytotoxic to A2780 and other ovarian cell lines, the sustained effects on culture growth raised suspicion that it might also be affecting the proliferation of surviving cells. To test this possibility, we analyzed whether resveratrol affects cell cycle progression. As shown in Fig. 2A, within 24 h, at a concentration of 50 μM, resveratrol caused cells to accumulate in S-phase. At 100 μM, the majority of cells instead accumulated in G0-G1 (Fig. 2A). Interestingly, we did not detect an increase in cells with hypodiploid DNA content, suggesting that apoptotic cell death was not induced. Consistent with our data, a concentration dependence to resveratrol’s effects on the cell cycle has been reported previously (10, 18, 24, 25).

To determine the reversibility of the growth arrest caused by resveratrol, A2780 cells were incubated with resveratrol (50 μM) for increasing amounts of time (0–48 h), after which the drug was
Results indicate that apoptotic pathways controlled by Bcl-2 and Bcl-xL (40%) expressing cells (Fig. 3A). A2780 cells stably transfected to express Bcl-2 (in pSFFVneo) and Bcl-xL (in pcDNA3). Unique transfected clones were isolated, and expression of proteins was verified by immunoblotting. Lane 1, A2780-pcDNA3; Lane 2, A2780-Bcl-xL; Lane 3, A2780-pSFFVneo; Lane 4, A2780-Bcl-2. B, cell death determined after 24 h of exposure to vehicle control (□), resveratrol (50 μM; [squlf]), or cisplatin (3 μM; □) of A2780 and overexpressing clones. *, statistical significance (P ≤ 0.05). Data represent the means; bars, SD.

Resveratrol-induced apoptosis results in the release of mitochondrial cytochrome c. A2780 cells were treated with cisplatin (3 μM; Lanes 1 and 2), DMSO (Lanes 3 and 4), and resveratrol (50 and 100 μM) in the presence or absence of z-VAD-fmk as indicated for 24 h (Lanes 5–12). Cells were harvested and separated into cytosolic (C) and mitochondria (M)-enriched fractions. Thirty μg of protein were resolved by SDS-PAGE and immunoblotted to detect cytochrome c (Cyt c) and β-tubulin. Resveratrol-induced apoptosis is not required for cell death. To determine whether resveratrol treatment results in formation of the Apaf-1/caspase-9 apoptosome, we used size exclud
sion chromatography to fractionate native cytosolic extracts from treated cells on the basis of molecular weight. Individual fractions from gel filtration chromatography were then analyzed for Apaf-1 and caspase-9 by immunoblotting. In the absence of resveratrol, Apaf-1 and caspase-9 were present in high molecular weight complexes consistent with their presence within the ~700 kDa to 1.4 MDa apoptosome complex (fractions 8–13, Fig. 5, B and C). Apoptosome complex-associated caspase-9 was processed, being detected as its cleaved caspase-9, which was apparently released from the Apaf-1 apoptosome.

To determine whether caspase-9 activity is necessary for resveratrol killing, cells were incubated with either z-LETD-fmk (a negative control) or z-LEHD-fmk, inhibitors of caspases -8 and -9, respectively, before resveratrol treatment. As seen in Fig. 5D, neither caspase inhibitor significantly reduced killing by resveratrol; resveratrol alone induced killing in 50% of cells, as compared with 41 and 39% in cells treated with resveratrol plus LETD or LEHD (P ≥ 0.05). These results suggest that although caspase-9 is activated, it is not required for resveratrol-induced cell death. Moreover, identical results were obtained using the pan-caspase inhibitor z-VAD-fmk (data not shown), indicating that caspase activity, in general, is not necessary for A2780 cell death in response to resveratrol.

**Resveratrol Induces Autophagy.** Despite cytochrome c release, apoptosis formation, and caspase-9 cleavage, the majority of resveratrol-treated A2780 cells do not display morphology typical of apoptosis. Resveratrol-induced cell death occurs independently of caspase activity, is not inhibited by Bcl-2 or Bcl-xL, and does not result in hypodiploid DNA content (see Fig. 2A). Moreover, as seen in Fig. 6, the majority of resveratrol-treated cells do not display morphology consistent with apoptosis. In particular, A2780 cells respond to 50 μM resveratrol, a concentration that kills 50–75% of cells, without cytoplasmic condensation, nuclear fragmentation, or blebbing (Fig. 6C), features typical of apoptosis (23). A2780 cell death induced by resveratrol is associated with a microscopic appearance different from the appearance of cells treated with 3 μM cisplatin, a concentration that also kills 50–75% of A2780 cells, with the expected morphological features of apoptosis (Fig. 6, compare C with B). Resveratrol-treated cells do not condense, nuclei remain intact, and the cytoplasm and cell surface develop a highly granular appearance. Bcl-xL–expressing clones are resistant to cisplatin-induced apoptosis as compared with control cells, although there are some typically apoptotic cells present after cisplatin treatment (Fig. 6, D compared to D). In contrast, Bcl-xL–expressing clones treated with resveratrol are similar in morphology to resveratrol-treated A2780-pcDNA3 cells, with a highly granular appearance to the cytoplasm and no evidence of apoptosis (Fig. 6, F and C). Overall, these morphological characteristics provide further support for the hypothesis that nonapoptotic death mechanisms can mediate the response to resveratrol.

TUNEL staining, a standard technique commonly applied to detect apoptosis, was performed on resveratrol (50 μM)-treated cells after 24 and 48 h. Resveratrol caused unequivocally positive TUNEL staining (data not shown). However, because transcription (31, 32), artificial DNA breakage, necrosis (33), and nonapoptotic programmed death (34, 35) each lead to DNA changes that give positive results on TUNEL, these findings were not helpful in further defining the mechanism of cell death. Electron microscopic characterization, which has been the gold standard for most precisely determining the mode of cell death, was next used to distinguish between these possibilities.

Nonapoptotic programmed cell death is principally attributed to autophagy (type II programmed cell death). Autophagy is a series of biochemical steps through which eukaryotic cells commit suicide by degrading their own cytoplasm and organelles through a process in which these components are engulfed and then digested in double membrane-bound vacuoles called autophagosomes (36). Examination of control cells using TEM revealed normal nuclear and mitochondrial morphology (Fig. 7, A and B). Resveratrol treatment resulted in the appearance of autophagocytic granules by 24 h (Fig. 7, C and D). After 48 h of resveratrol treatment, cells undergoing autophagic cell death retained an intact nuclear membrane, without chromatin condensation. Autophagocytic granules contained extensively degraded organelles (Fig. 7, E and F). Cells treated with cisplatin showed...
characteristic changes associated with apoptosis, including intact cellular membranes, blebs at the cellular surface consistent with the formation of apoptotic bodies, and chromatin condensation, in the absence of autophagocytic bodies (Fig. 7, G and H).

To confirm that resveratrol-induced cell death was largely non-apoptotic, nuclear morphology was evaluated using Hoechst staining. A2780 ovarian cancer cells were treated with DMSO solvent control, resveratrol, or cisplatin. The nuclei of resveratrol-treated cells appeared similar to control DMSO-treated cells. In contrast, cisplatin treatment resulted in the appearance of clumped, condensed chromatin, along with a disintegrating nuclear envelope (Fig. 8, A–C). MDC, a fluorescent compound selectively taken up by autophagosomes, was used to obtain independent evidence supporting the conclusion that resveratrol triggers autophagocytosis (37). MDC was applied to A2780 cells after resveratrol treatment, and when these labeled cells were imaged using epifluorescence microscopy, treated cells demonstrated an intense, punctate fluorescence pattern (Fig. 8E). In contrast, control cells had minimal fluorescence (Fig. 8D). To further implicate autophagocytosis, we planned to pretreat A2780 cells with the phosphoinositide kinase inhibitor, 3-MA, known to block autophagocytic signaling (38). Unfortunately, appropriate concentrations of 3-MA alone killed these cells, precluding the use of this agent as intended (data not shown).

DISCUSSION

Resveratrol is a phytoalexin that helps protect plants against pathogens (39). Specific anticancer effects of resveratrol have also been shown in vitro and in vivo. The most persuasive evidence shows that resveratrol affects early steps in the process of carcinogenesis. It inhibits the formation of neoplastic skin and mammary lesions (14, 40), it blocks transformation of rat epithelial cells by N-nitrosomethylbenzylamine (41), and it suppresses gastrointestinal tumor formation in Min mice (42).

Resveratrol may also limit the survival and proliferation of cancer cells, more consistent with the actions of a conventional chemotherapeutic drug. Its influence on cellular redox balance, inhibitory effects on estrogen hormone signaling, and antiangiogenic functions may all be relevant for its effects on the late stages of carcinogenesis. Resveratrol’s antiproliferative and apoptosis-inducing capabilities have been demonstrated in many cell types (43). Its biochemical actions are coupled to multiple signaling pathways, such as nuclear factor-κB, cyclo-oxygenase-2, and inducible nitric oxide synthase expression, and it changes the levels of several components of the cyclin-dependent kinase system (44–48). Nevertheless, outside of a few early reports, there is disappointingly little conclusive evidence that demonstrates its effectiveness at treating already existing tumors or malignancies in animals (8, 49, 50).
In this study, we found that resveratrol inhibits the proliferation and survival of five malignant ovarian carcinoma cell lines. Cell cycle analysis demonstrated that resveratrol induces cell cycle arrest with the following features: after 12 h of exposure, removing the drug does not reverse growth arrest; moreover, cell cycle arrest is concentration dependent such that lower concentrations induce arrest at the G1/M transition with accumulation in the S-phase, whereas concentrations >50 μM cause accumulation of cells in G2/M (see Fig. 2). These results are consistent with other reports showing that the antiproliferative effects of resveratrol are variably linked to either S-phase arrest, such as in MCF7 and HL60 cells, or G1 arrest in A431 epidermoid carcinoma cells (25, 51).

We observed dose-dependent cytotoxic effects of resveratrol against each of the ovarian cell lines. Cell death, determined by plasma membrane permeability changes, is maximal by 48 h of treatment. We fully characterized cell death in A2780 cells, which, among the cell lines tested, was the most sensitive to resveratrol. Interestingly, although the apoptosome complex forms as an early response to treatment, cell death also occurs in the presence of apoptotic inhibitors, indicating that resveratrol can activate nonapoptotic cell death. Resveratrol induced cell death is independent of caspase function or Bcl-2/Bcl-xL expression, cellular DNA is not degraded, and the resulting cellular morphology is not typical of apoptotic cell death. Resveratrol induced cell death is independent of type II programmed cell death. Caspase function or Bcl-2/Bcl-xL expression does not influence cell death, and the resulting cellular morphology is not typical of apoptosis. Rather, our results point to autophagocytosis (autophagy or type II programmed cell death) as the main mode of death.

The involvement of an autophagocytic mechanism explains why particular cells that are resistant to cisplatin and other proapoptotic drugs, for example Bcl-2- and Bcl-xL-expressing A2780 clones, or ES-2 cells, are sensitive to resveratrol. Resistance to apoptosis is a significant problem in ovarian cancer treatment and in some cases is understood to result from tumor cell defects in apoptosome function or from the expression of high levels of Bcl-2 or other antiapoptotic genes. Consequently, agents such as resveratrol, which are capable of killing by an alternative, nonapoptotic death mechanism, have considerable appeal as experimental agents against this disease. Along these lines, it is interesting to note that a previous study with human leukemia U937 cells found that high-level Bcl-2 attenuated resveratrol-induced cell death (apoptosis; Ref. 52). Therefore, resveratrol’s ability to trigger autophagocytic death and overcome failed apoptosis may depend on the particular cell type involved.

Autophagy is a physiological mechanism that involves the sequestration of cytoplasm and intracellular organelles into membrane vacuoles called autophagosomes and results in their eventual enzymatic degradation (36). In response to appropriate stimulation, depolarized mitochondria are known to move into autophagic vacuoles. Thus, mitochondrial dysfunction may be a point of overlap between apoptotic and autophagic processes (53). The fusion of the edges of the membrane sac forms a closed double-membrane structure, the so-called autophagosome. Finally, the autophagosome fuses with a lysosome to become the autolysosome. Within autolysosomes, lysosomal hydrolases degrade the sequestered cellular constituents.

A2780 cells treated with resveratrol demonstrate an ultrastructural appearance consistent with the formation of autophagosomes. Furthermore, resveratrol-treated cells stain with MDC, a specific marker for autophagic vacuoles (37). Confirmatory experiments were performed using 3-MA to block autophagocytosis (54). In our experiments, however, 3-MA alone was toxic at appropriate concentrations, precluding an attempt to determine its capacity to block resveratrol killing (data not shown).

Adding autophagy to the list of resveratrol’s bioactivities has implications for cancer treatment and chemoprevention. We have shown that although resveratrol-induced cell death can trigger formation of the apoptosome and apoptosis, an alternative pathway of cell death, autophagocytosis is also activated (see model, Fig. 8). Gene products that regulate autophagy also function as tumor suppressor genes, supporting the argument that this process is involved in the elimination of cancer cells by triggering a nonapoptotic cell death program (53, 55). One particularly interesting example is Beclin 1, a Bcl-2-interacting coiled-coil protein. Beclin 1 promotes autophagy when overexpressed in MCF-7 cells, and beclin-1 is monoaellically deleted in 40–75% of sporadic breast and ovarian cancers (56). Furthermore, treatment of MCF-7 cells with tamoxifen, a widely used preventive and therapeutic agent for breast cancer, has been shown to induce autophagocytosis (57). Experimentation is planned to determine whether resveratrol-induced death depends on beclin-1 or other specific gene products thus far implicated in human cell autophagocytosis.

One practical question with resveratrol is whether serum or tissue concentrations can be attained that reach the levels at which growth arrest and autophagy are observed in vitro. In humans, there is an insufficient understanding of the pharmacokinetics and bioactivity of resveratrol and its metabolites to know this with certainty. In rats receiving an oral dose of 2 mg/kg, peak concentrations of 2.6 μM were achieved. Resveratrol is extensively glucuronidated and sulfated in the liver and is converted by the P450 isoenzyme CYP1B1 to piceatannol, which has antitumor cell properties of its own (58). For example, when resveratrol was given to mice as a 60-mg/kg oral dose, serum levels of resveratrol-glucuronide and sulfate exceeded 100 and 300 μM, respectively (59). Although the bioactivity of these conjugated metabolites has not been tested, their hydrophilic properties make it unlikely that either will be accessible to intracellular targets. Nevertheless, β-glucuronidase activity is present at high levels in the extracellular space of certain bulky tumors, and enzymes with sulfatase activity are expressed at particularly high levels in gynecological tumors, including ovarian cancers (60, 61). These enzymes may be capable of converting the resveratrol metabolites back to trans-resveratrol, providing tumor-selective bioactivation and a sufficient concentration of active drug to induce autophagy.

Therapeutic utility of anticancer drugs is largely governed by selectivity against diseased tissue and tolerability of side effects relative to overall benefit. In animal models, resveratrol’s toxicity is minimal, and even actively proliferating tissues are not adversely affected (e.g., bone marrow, gastrointestinal tract). The maximum tolerated dose of resveratrol in mice is 4,000 mg/kg/day for 28 days. Indeed, the minimal toxicity in animals has allowed resveratrol to enter human studies sponsored by the National Cancer Institute aimed at cancer prevention using healthy volunteers. We are beginning studies to determine whether resveratrol is active against ovarian cancer xenografts in mice to assess the degree to which the compound selectively targets tumor cells relative to normal tissues and to show whether autophagy is observed in vivo.

In summary, we have identified a novel activity for resveratrol in ovarian cancer cells, the ability to induce autophagocytosis. This response and its ability to growth arrest these cells hint at the possibility that this agent may be useful as an adjuvant therapy to treat ovarian tumors. Although resveratrol treatment engages certain components of the apoptotic machinery, important inhibitors of apoptosis, such as Bcl-2, do not limit its cytotoxic effects on these cells. These findings pave the way for additional experiments to consider the molecular basis for this response, a potential role for beclin-1, and studies to determine whether adequate concentrations of bioactive resveratrol can be attained to treat ovarian tumors.

4 Dr. Tristan Booth, Royal Mount Pharma, personal communication.
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