RESEARCH COMMUNICATION

Resveratrol Exerts Differential Effects *in Vitro* and *in Vivo* against Ovarian Cancer Cells

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Abstract

Epithelial ovarian cancer represents the most lethal gynecological cancer, and the high mortality rate makes this malignancy a major health concern. Poor prognosis results from an inability to detect ovarian cancers at an early, curable stage, as well as from the lack of an effective therapy. Thus, effective and novel strategies for prevention and treatment with non-toxic agents merit serious consideration. Resveratrol, obtained from grapes, berries, peanuts and red wine, has been shown to have a potent growth-inhibitory effect against various human cancer cells as well as in *in vivo* preclinical cancer models. The objective here was to evaluate potential antitumor effects of resveratrol in both *in vitro* and *in vivo* NuTu-19 ovarian cancer models. *In vitro* an invasion assay was performed. After 48 h, the numbers of viable cells that invaded the extracellular matrix layer were reduced by 94% with resveratrol in comparison to control. For the *in vivo* anti-tumor assessment, 10 rats were injected with NuTu-19 cells into the ovarian bursa. Thereafter, half were provided with a diet mixed with a dose of 100 mg resveratrol/kg body weight/day for 28 days. Following sacrifice, anticancer effects were assessed by histological evaluation of ovarian as well as surrounding tissues, and immunohistochemical detection of cell proliferation and apoptosis, but there were no observable differences between the control and resveratrol-treated groups for any of the biological endpoints. While resveratrol is effective in suppressing the *in vitro* cellular invasion of NuTu-19 ovarian cancer cells, these effects do not appear to impact on *in vivo* NuTu-19 ovarian cancers in rats.

Keywords: Resveratrol - NuTu-19 cells - ovarian cancer - anti-invasion effects - chemoprevention

Asian Pacific J Cancer Prev, **13**, 1333-1340

Introduction

Ovarian cancer represents the second most common malignancy of the female genital tract and fifth leading cause of cancer deaths in the female population throughout the world (Sankaranarayanan et al., 2006). It is the most lethal gynecologic malignancy with more than 140,000 deaths occurring in women worldwide. Nearly 22,300 new cases of ovarian cancer and approximately 15,500 deaths were estimated to occur in the United States in 2012 (Siegel et al., 2012). The high mortality rate arises primarily from the lack of an effective screening approach combined with inadequate therapeutic options for the disease in its advanced stages. Despite improved survival for many malignancies in clinical oncology, the 5-year relative survival rate for patients with advanced stage ovarian cancer remains low (~30%) and has essentially been the same over the past two decades (Visintin et al., 2008). Accordingly, the National Cancer Institute identified the need for defining treatment strategies for ovarian carcinoma (NIH, 1995). In view of the slow pace of progress in the treatment of advanced ovarian cancer, the quest for strategies for prevention as well as novel effective therapy hold significant promise to reduce the mortality from this deadly disease (Mills, 2002; Brewer et al., 2003; Hoekstra & Rodriguez, 2009). A plethora of phytochemicals present in vegetables, fruits, nuts and spices has been shown to be effective in the prevention and treatment of several tumors (Stan et al., 2008; Dennis et al., 2009; Gullet et al., 2010; Bishayee, 2012). Some are currently being considered as promising agents in the prevention and management ovarian cancer (Hoekstra & Rodriguez, 2009).

Resveratrol (3,4',5-trihydroxy-*trans*-stilbene, $C_{14}H_{12}O_3$, molecular weight 228.2, Figure 1) is a natural

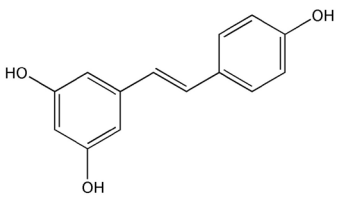

Figure 1. Resveratrol Chemical Structure

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phytoalexin synthesized in more than 70 plant species in response to injury, UV radiation, and insect or fungal attack. Because of its high concentration in grape skin, a significant amount of resveratrol is present in wines, especially red wines, and it is considered to be responsible for the beneficial effects of red wines against coronary heart disease (Fremont, 2000). Resveratrol is gaining tremendous importance as it possesses cancer preventive as well as anticancer activities in various biological systems (reviewed by Bishayee, 2009). In animal studies, resveratrol prevents or delays the development of skin (Jang et al., 1997), mammary (Banerjee et al., 2002) and prostate (Harper et al., 2007) tumors as well as blocks or suppresses esophageal (Li et al., 2002), gastric (Zhou et al., 2005), small intestinal (Schneider et al., 2001), colonic (Tessitore at al., 2000; Sengottuvelan et al., 2006) pancreatic (Harikumar et al., 2010), and hepatic (Bishayee & Dhir, 2009) tumorigenesis. Resveratrol has also been shown to possess *in vitro* cytotoxic effects against a wide variety of human tumor cells, including lymphoid and myeloid cancer cells as well as skin, breast, ovary, cervix, prostate, stomach, colon, pancreas, liver and thyroid carcinoma cells (reviewed by Aggarwal et al., 2004; Kundu and Surh, 2008).

Resveratrol displays pleiotropic effects and has a multitude of biochemical and molecular actions including inhibition of free radical formation and activities of cyclooxygenase (COX), inducible nitric oxide synthase, cytochrome P-450 and protein kinase C, and directly binds to estrogen receptors and the F1 component of mitochondrial ATP synthase. Cell proliferation and DNA synthesis is decreased through its inhibitory actions on ribonucleotide reductase, DNA polymerase, and ornithine decarboxylase activities as well as by cell cycle arrest at the G2-S checkpoint. Apoptosis is induced in various malignant cells by resveratrol through up-regulation of CD95L expression, caspase activation, stabilization of p53, and inhibition of nuclear factor κB activity (Athar et al., 2009; Delmas et al., 2011; Shukla and Singh, 2011). Several pathways are intertwined and influence additional mechanisms involved in cellular invasion and metastasis, such as the regulation of growth factors and matrix metalloproteins (Weng & Yen, 2012). These multiple overlapping mechanisms contribute to the overall impact of resveratrol's effects against precancerous or cancer cells.

Resveratrol possesses tremendous potential for ovarian cancer chemoprevention and treatment due to the aforementioned biochemical and molecular effects as well as its minimal toxicity. Moreover, specific inhibitors of COX-2 have been found to have chemopreventive action in a number of epithelial cancers, including colon, mammary glands, esophageal, lung and oral cavity (Subbaramaiah et al., 1997). Recently, it has been hypothesized that by inhibiting COX-2, the loss of the basement membrane of the ovarian surface epithelium may be lessened, and consequently the neoplastic transformation of the ovarian surface epithelial cells may be reduced (Smith et al., 2004). However, a study for the use of resveratrol, a COX-2 inhibitor, for chemoprevention of ovarian cancer has not been performed and thus merits immediate attention.

Resistance of recurrent disease to cytotoxic drugs has been the principal factor limiting long-term success against ovarian cancer. The oncogenesis of ovarian cancer appears to favor the development and subsequent expansion of cell clones that are resistant to apoptic triggers. An important clinical prospect is to identify lead compounds that circumvent the resistance mechanisms that limit the success of conventional drugs. Opipari and colleagues (2004) has shown that resveratrol induces death in ovarian cancer cells by autophagocytosis, a mechanism distinct from apoptosis, suggesting that it may provide leverage to treat ovarian cancer that is chemoresistant on the basis of ineffective apoptosis. Further, alterations in the angiogenic characteristics of ovarian surface epithelium may play an important role in the etiology of ovarian cancer (Schumacher et al., 2007). Human ovarian cancer progression and angiogenesis were reduced by resveratrol through suppression of hypoxia-inducible factor 1α (HIF-1α) and vascular endothelial growth factor (VEGF) as observed (Cao et al., 2004). Thus, the potential array of therapeutic targets for this remarkable natural compound implicates the novel rationale for resveratrol as an extremely attractive candidate in the treatment of ovarian cancer.

Even though numerous animal tumor models have been developed to aid in the research of human ovarian cancer, a strong need exists for an animal model that allows research in early-stage ovarian cancer and progression of disease (Sloan Stakleff & Von Gruenigen, 2003). Epithelial ovarian cancer does not develop spontaneously in rodents to the extent necessary to support studies of chemoprevention and treatment. A novel early-stage orthotopic ovarian cancer model in Fischer 344 rats has been developed by Sloan Stakleff and coworkers (Sloan Stakleff et al., 2005) which emulates early disease with the initiation of a primary tumor localized within the inherent microenvironment. This animal model offers a clinically relevant alternative for cancer research that allows for the investigation of therapeutic strategies against early stages of the disease process.

The objectives of the present study were to evaluate the anti-invasive efficacy of resveratrol against NuTu-19 ovarian cancer cells as well as anti-tumor, anti-proliferative and apoptosis-inducing potential of this natural product against an early stage orthotopic model of ovarian cancer in rats.

Materials and Methods

Resveratrol

Trans-resveratrol was received from Pharmascience Inc. (Montréal, Canada) as a generous gift. It was synthesized using good manufacturing practice and tested at 98-101% purity (in anhydrous and solvent free basis) by high performance liquid chromatography.

In vitro invasion assay

NuTu-19 ovarian cancer cells were originally obtained as a dormant culture from Dr G Scott Rose (Walter Reed Army Medical Center, Washington, DC). Cell cultures were maintained through serial passage as described in

previous communications (Sloan et al., 2005; Medvetz et al., 2007). *In vitro* cellular invasion assay was performed as previously described (Medvetz et al., 2007). Briefly, NuTu-19 cells (105 cells) were suspended in Matrigel (85 μg/cm2, Becton Dickenson, Franklin Lakes, NJ) and layered onto porous polycarbonate filters (8 μm, 0.33 cm^2) in Transwell invasion chambers (Costar, Corning, Lowell, MA). Resveratrol was initially dissolved in dimethylsulfoxide (DMSO) and subsequently diluted with the culture media for treatment of cells at 100 μM concentration while maintaining the concentration of DMSO at 0.05%. The control cells were exposed to 0.05% DMSO in media. After 48 hours, cells that traverse the membrane to the undersurface and outer well were released with trypsin-EDTA and collected. The numbers of viable cells that invade the extracellular matrix layer were counted by trypan blue exclusion.

In vivo ovarian cancer study

Female Fischer 344 rats were obtained from Harlan Laboratories (Indianapolis, IN). All procedures involving animals were performed in compliance with the guidelines of the Institutional Animal Care and Use Committee of the Akron General Medical Center (Akron, OH) and the Northeast Ohio Medical University (Rootstown, OH). Ten rats were divided between the control and resveratrol treatment groups with 5 animals each. NuTu-19 cell suspensions were prepared in serum-free media containing 104 cells per 5 μl. Cells were injected below the ovarian bursa according to published technique (Sloan et al., 2005). In the treatment group, the rats had free access to a pulverized feed supplemented with trans-resveratrol equivalent to 100 mg/kg body weight/day for 28 days. The dose of resveratrol has been calculated based on the average daily food intake. The oral route of administration as well as the dose (100 mg/kg) of resveratrol was selected based on the *in vivo* evidence of the efficacy of resveratrol in inhibiting hepatic tumorigenesis in rats without any toxic manifestations (Bishayee & Dhir, 2009). Additionally, no adverse effects have been found for a dose up to 300 mg resveratrol/kg/day for 4 weeks in rats as per the studies conducted by other investigators (Crowell et al., 2004; Hebbar et al., 2005). Food and water intake as well as behavioral changes were monitored every day and body weight of animals recorded every week over 28 days.

Histological and histochemical analyses

At the end of the study, animals were euthanized by CO_2 asphyxiation. Ovarian tissue was excised, weighed, fixed in 10% neutral-buffered formalin, and embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin and eosin and subsequently examined for evidence of microscopic metastases. Presence of glycoproteins as a marker of epithelial-derived ovarian cancer was detected by staining tissue sections using periodic acid-Schiff (PAS) reaction (Aalto & Collan, 1986). All PAS staining reagents were obtained from Sigma-Aldrich, (St. Louis, MO). Stained slides were compared to diastase-digested sections to identify glycoprotein-cleared regions. All slides were coded so that the particular treatment was unknown to the individual performing the assessment.

Cell proliferation

Immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in formalin-fixed, paraffin embedded tumor sections (5 μm) was performed by the modified streptavidin-avidin-biotin-immunoperoxidase-complex method of Hsu et al. (1981) as previously described (Bishayee & Dhir, 2009). Briefly, following the removal of paraffin, the endogenous peroxidase was blocked by 1% H_2O_2. Then the samples were incubated in 3% bovine serum albumin for 30 min to prevent unspecific reaction followed by incubation with primary anti-PCNA antibody (Santa Cruz Biotechnology, Santa Cruz, CA). PCNA-positive nuclei were identified by alkaline phosphatase labeled streptavidin-biotin complex (Invitrogen, Camarillo, CA). The sections were counterstained with hematoxylin and examined for the presence of brownish yellow particles covering the nuclei to represent the positive signal of PCNA.

Apoptosis

Tumor sections were assessed for apoptosis using a terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay. Fluorescein-12-dUTP was catalytically incorporated into the 3'-OH DNA ends of apoptic nuclei using the Promega DeadEnd Fluorometric TUNEL System (Madison, WI) following the manufacturer's instructions. Three serial sections from each tissue (from control and resveratrol-treated rats) were used in order to evaluate the fluorescence from positive (DNase added to fragment the DNA) and negative (no DNase/no transferase) assay controls as well as the experimental slide.

Expression of results and statistical significance

Results for the *in vitro* invasion assays are presented as mean ± standard error (SE) for triplicate wells with the experiment in duplicate. Statistical analyses were performed using Student's *t*-test, and a value of p less than 0.05 will be considered significant.

Results

Effects of resveratrol on the invasion of NuTu-19 cells

The invasive capacity of the NuTu-19 cells was substantially inhibited by 94% with the resveratrol treatment (100 μM) for 48 h. The numbers of NuTu-19 cells that were able to invade across the Matrigel membrane were significantly ($p<0.05$) reduced in the wells treated with resveratrol in comparison to the control wells. The results from the in vitro experiment substantiated the progression to examine the effects of resveratrol treatment in the *in vivo* NuTu-19 ovarian cancer model.

Effects of resveratrol on ovarian tumor and lung metastases

All ten animals were sacrificed 28 days post-injection of NuTu-19 cells. Upon necropsy, the animals were examined for evidence of ovarian tumor growth. Tumor was grossly identified in only one of the resveratrol-treated

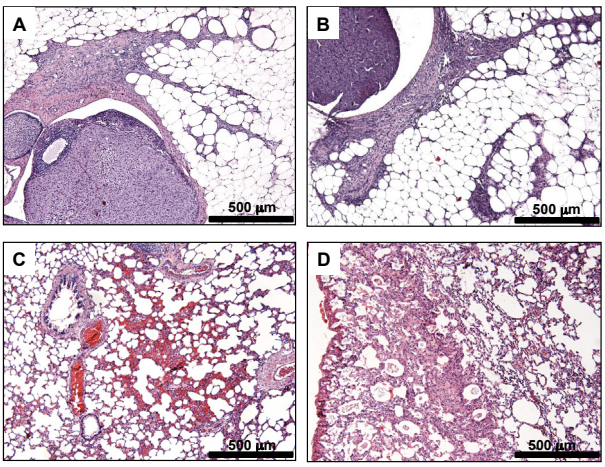

Figure 2. Histological Examination of Ovarian Tissue and Lung Specimens Stained by Hematoxylin and Eosin. Primary tumor cells were observed as protruding from the ovarian capsule and extending into the omentum for both control (A) and resveratrol-treated (B) animals. Metastatic tumor cells located in the lungs were radiating from the blood vessels in the control group (C) and diffusely infiltrating the capsule in the resveratrol-treated groups (D). Magnification: 100X.

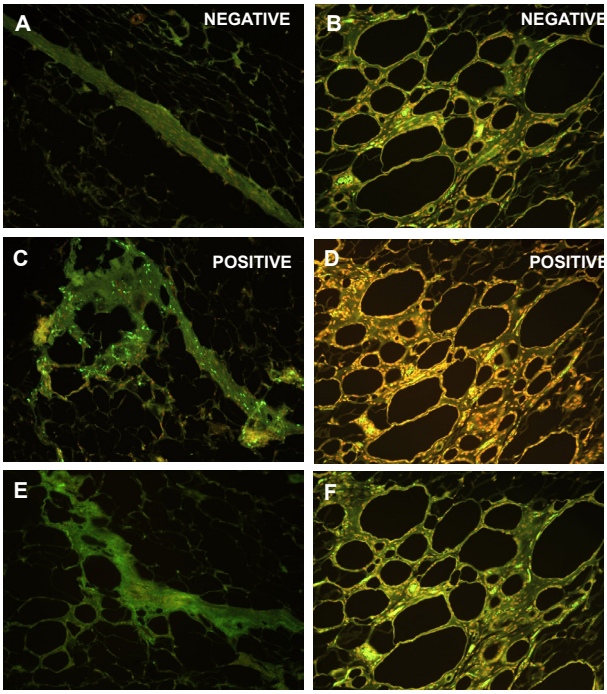

Figure 3. Effects of Resveratrol on Apoptosis. Positive slides (C and D) displayed enhanced fluorescence on the ovarian cancer cells in the regions of the tumor located within the stroma or omentum. Control (E) and resveratrol-treated group (F) showed identical fluorescence patterns to that observed in the respective negative slides (A and B, respectively)

rats and lesions suspicious of metastases were observed on the lungs of two animals in each group (see Figure 2). However, microscopic examination of the ovary specimens revealed the presence of microscopic tumor cells in the control as well as resveratrol-treated animals and metastases were confirmed in the lungs of animals with or without resveratrol exposure.

Effects of resveratrol on glycoproteins

Ovarian sections from both the control as well as resveratrol-treated group were stained using PAS to detect the presence of glycoproteins that are typically increased in epithelial-derived cancers, including ovarian cancer (Aalto & Collan, 1986). For each group, the PAS-stained slides were compared to the corresponding serial sections digested with diastase to identify the glycogen-cleared regions. Nevertheless, no differences were noted between the PAS and PAS-diastase slides in the regions containing NuTu-19 ovarian cancer cells in control animals. These results demonstrate that NuTu-19 ovarian cancer cells do not upregulate the production of glycoproteins. Similarly, resveratrol treatment did not modify the status of glycoproteins in animals injected with NuTu-19 cells.

Effects of resveratrol on cell proliferation

Tissue sections were stained for PCNA immunoreactivity with expectation that the mitotically active NuTu-19 ovarian cancer cells would express the antigen. PCNA-positive staining was not detected in the tissues from control and resveratrol treated-animals. The lack of staining may be attributed to fixation methods or the necessity to employ additional antigen retrieval methods. The limited amount of tissue available for the histological panel of analyses prohibited further assays to explore these options.

Effects of resveratrol on apoptosis

As apoptosis-inducing properties of resveratrol have been implicated in its anti-tumor efficacy (Delmas et al., 2011), induction of apoptosis was anticipated in the ovarian tissue from NuTu-19 cells injected animals treated with resveratrol. The TUNEL assay was performed on triplicate serial tissue sections from the control and resveratrol-treated groups. For each group, the test slide was compared to the positive and negative assay control slides. Negative slides (Figure 3) demonstrated typical autofluorescence of blood cells within vascular structures; while the positive slides (C and D) displayed enhanced fluorescence on the ovarian cancer cells in the regions of the tumor located within the stroma or omentum. Evaluation of the tissue sections from the control (E) and resveratrol-treated group (F) showed identical fluorescence patterns to that observed in the respective negative slides (A and B, respectively).

Discussion

Review of the recent literature provides a convincing body of evidence demonstrating resveratrol to have numerous antitumor properties including the ability to decrease cell proliferation, induce apoptosis, and inhibit angiogenesis and tumor growth by mediating a variety of cellular pathways (Bishayee, 2009; Athar et al., 2009; Weng & Yen, 2012). These effects have been documented in numerous types of cancer cells from skin, breast, ovary, prostate, gastrointestinal tract and lung (Aggarwal et al., 2004; Kundu & Surh, 2008). Based on these findings in epithelial-derived cancers, the present study was designed to assess the efficacy of resveratrol in the treatment of ovarian cancer.

The *in vitro* cellular invasion assay produced encouraging results when resveratrol significantly

suppressed the invasion of the NuTu-19 ovarian cancer cells across the Matrigel membrane compared to untreated control cells. Several studies have previously demonstrated marked antitumor activities of resveratrol against various ovarian cancer cells. Resveratrol has been shown to inhibit both the growth and proliferation of human ovarian cancer cells, such as OVCAR-3, PA-1 and SKOV-3, through induction of apoptosis, DNA damage and S-phase arrest (Yang et al., 2003; Tyagi et al., 2005; Raj et al., 2008; Lee et al., 2009; Björklund et al., 2011; Lin et al., 2011; Marimuthu et al., 2011). Resveratrol induced cell death and growth inhibition in A1947, A2780, CaOV3, ES-2, SKOV3 and TOV112D cells by autophagocytosis and inhibition of glycolysis (Opipari et al., 2004; Kueck et al., 2007). Abrogation of HIF-1α and VEGF has been implicated in resveratrol-mediated inhibition of cell migration and angiogenesis in A2780/CP80, CAOV-3 and ovcar-3 cells (Cao et al., 2004; Park et al., 2007). Resveratrol has also been shown to augment the growth inhibitory effects of other phytoantioxidant, namely indole-3 carbinol (Raj et al., 2008) as well as standard chemotherapeutic drugs, such as cisplatin and doxorubicin (Björklund et al., 2011; Rezk et al., 2006). Our *in vitro* results represent the first experimental evidence of anti-invasive activity of resveratrol against NuTu-19 ovarian cancer cells supporting the potential of this bioactive natural product in treating ovarian cancer.

Our *in vitro* results supported the pursuit of investigating the anti-tumor efficacy of resveratrol against an *in vivo* animal ovarian cancer model. A novel, clinically relevant early-stage ovarian cancer model developed by Sloan Stakleff et al. (2005) was utilized in this study. The model was designed by a unique approach of combining surgical orthotopic implantation techniques with the syngeneic NuTu-19 tumor cells and immunocompetent Fischer 344 rats. NuTu-19 tumor cells derived from ovarian surface epithelium of the Fischer 344 rats provide a syngeneic model with complete immunologic compatibility between the cells and the animal (Rose et al., 1996). The ovary was surgically isolated and NuTu-19 cells were directly injected below the epithelial bursa surrounding the ovary. The orthotopic injection initiates the growth of a primary tumor within its inherent cellular microenvironment and encompasses the three general stages of neoplasia, namely initiation, promotion, and progression. The orthotopic animal model clearly represents the full spectrum of progression of human disease by creating a localized primary lesion (stage I), pelvic and abdominal adhesions with pelvic implants (stage II), and metastatic spread to peritoneal organs (stages III and IV) [36].

In our study, rats injected with NuTu-19 cells were exposed to dietary resveratrol for 4 weeks. Following this treatment period, tissue specimens were collected and prepared for histological staining by standard and specialized methods. Hematoxylin and eosin staining was performed to locate the NuTu-19 ovarian cancer and the metastatic progression into the omentum as well as lung. Our results did not show any modifying effects of resveratrol on the growth of NuTu-19 cells in ovary and migration of metastatic cancer cells in the omentum and lung. The PAS stain was performed to examine whether NuTu-19 cells display an increase in glycoprotein production as typically observed for ovarian (Aalto & Collan, 1986) and other epithelial-derived cancers. The absence of PAS positivity in control and resveratrol-treated animals indicates glycoproteins are not vital for NuTu-19 ovarian cancer cell growth and metastasis.

The objective of the immunohistochemical PCNA and fluorometric TUNEL analyses was primarily to determine the effects of resveratrol on cell proliferation and apoptosis during the progression of NuTu-19 ovarian cancer. We did not observe any difference in the PCNA expression in ovarian tissue injected with NuTu-19 cells in rats treated with resveratrol and control animals. Although NuTu-19 ovarian cancer cells were shown to be apoptosis resistant, these cells can be induced to undergo apoptosis when tumor-associated cellular mechanisms are stabilized (Feki et al., 2005; Brard et al., 2006). Since resveratrol mediates effects on a range of pathways (Athar et al., 2009; Bishayee, 2009; Weng & Yen, 2012), cellular stabilization and apoptosis was expected. However, regions of ovarian cancer from control and resveratrol-treated animals showed no detectable difference in fluorescence compared to the negative controls, indicating the absence of resveratrol-mediated apoptosis in the NuTu-19 cells in an in vivo condition.

Despite the study began with the promising in vitro invasion assay results, the *in vivo* study overall showed no observed differences between the resveratrol and control groups in any of the parameters that were assessed. There are only two other *in vivo* studies in which anti-ovarian cancer activities of resveratrol have been tested. According the study conducted by Lee and colleagues (Lee et al., 2009), intraperitoneal (i.p.) administration of resveratrol (50 or 100 mg/kg, once daily for 4 weeks) in female Balb/c (nu/nu) mice xenografted with PA-1 cells, starting the treatment 10 days following tumor cell implantation, retarded the growth of tumor with simultaneous decrease in cell proliferation and apoptosis. In another study, resveratrol or resveratrol-bovine serum albumin nanoparticle preparation (50, 100 or 200 mg/kg; once a week for 4 weeks), administered through i.p. injections beginning on the 7th day post-implantation, significantly inhibited the growth of xenografted SKOV3 carcinoma in nu/nu mice via apoptosis regulated through the mitochondrial apoptotic pathway (Guo et al., 2010). Although a tumor xenograft model may not be readily interpreted as the true physiologic or clinical condition (Gescher & Steward, 2003; Becher & Holland, 2006; Teicher, 2006), our results may indicate differential response of resveratrol on orthotopic ovarian cancer as compared to xenograft models.

To our knowledge, there are currently no reports on the effects of resveratrol on NuTu-19 cells or a syngeneic, immunocompetent, physiologic ovarian cancer animal model. Apart from the differences in immune status and cells used, other potential explanations for the disparity in results between the xenograft and syngeneic *in vivo* models may pertain to the route of administration (i.p. versus oral) as well as the metabolism of resveratrol. The concentration of biochemically active resveratrol reaching the target tissues may vary greatly with oral ingestion

compared to the i.p. injection.

Resveratrol is rapidly metabolized by the liver and intestines into glucuronidated and sulfated compounds and these metabolites have not been shown to inhibit tumor cell growth (Subramanian et al., 2010). Since resveratrol is primarily metabolized in the liver, the gastrointestinal bioavailability would be significantly thus explaining the demonstrated efficacy of resveratrol in gastrointestinal cancers, including liver cancer (reviewed by Bishayee, 2009). The low circulating levels of drug reaching the ovarian cancer tissue may be too low to yield a therapeutic threshold to achieve an effect in the current study. In view of these, efficacy of increasing doses of resveratrol would be investigated in future experiments.

In reviewing the outcomes of this study as well as other reports available in literature, it appears that evidence may be mounting to suggest that there is a disparity in the activity of resveratrol *in vitro* versus *in vivo*. Bove et al. (2001) reported that resveratrol inhibited the growth of 4T1 breast cancer cells in vitro but had no effect on tumor growth or metastatic spread of the same cancer cells in an *in vivo* xenograft model following i.p. administration for 23 days starting at the same time of tumor cell inoculation. Similarly, dietary resveratrol did not affect the growth of xenografted A375 human melanoma cells in mice (Niles et al., 2006) in spite of inhibiting the growth of the same malignant cells in vitro (Niles et al., 2003). The aforementioned studies indicate that the *in vivo* anti-tumor effects of resveratrol could be dependent on the experimental models used.

In conclusion, the results of the anti-invasion effects of resveratrol against NuTu-19 ovarian cancer cells represent a promising observation. Nevertheless, resveratrol did not show a clear anti-tumor effect against an early stage rat orthotopic model for ovarian cancer utilizing the same cancer cells. Our *in vitro* data demonstrating the anti-invasive properties coupled with results on *in vitro* and *in vivo* anti-cancer effects of resveratrol against diverse ovarian cancer models as reported from several laboratories underline the potential of this dietary phytochemical in prevention and treatment of chemotherapy-resistant ovarian cancer. Future possibilities may include a different route of administration or formulation, which might be more effective in this particular type of cancer. The poor oral bioavailability of resveratrol is likely the greatest contributing factor. Therefore, developing derivatives of resveratrol that have enhanced bioavailability is a rational approach to achieve a similar *in vivo* outcome as compared to what has been achieved *in vitro*.

Acknowledgements

This work was supported by a pilot research grant from Akron General Medical Center Development Foundation (Akron, OH) to Anupam Bishayee, Ph.D. The funding source had no involvement in the study design, collection, analysis and interpretation of the data, writing of the report, and decision to submit the manuscript for publication. The authors sincerely thank Thomas Mbimba and Heather Lentz for their assistance with histopathological and immunohistochemical studies and Altaf S. Darvesh, Ph.D., for critical reading of the manuscript. Tristan D. Booth, Ph.D., is an employee of Pharmascience, Inc., Montréal, Québec, Canada. The other authors declare that they have no conflicts of interest.

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