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Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts in vivo

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Abstract

Resveratrol, a polyphenol found in grapes and wine, is considered a potential cancer chemopreventive agent. Resveratrol has been shown to induce transcription via both ERα and ERβ. We observed significantly lower tumor growth, decreased angiogenesis, and increased apoptotic index in ERα− ERβ+ MDA-MB-231 tumors in resveratrol-treated nude mice compared with controls. In vitro we found a significant increase in apoptosis in resveratrol-treated MDA-MB-231 cells in addition to significantly reduced extracellular levels of VEGF. This study supports the potential use of resveratrol as a chemotherapeutic agent in breast cancers.

Keywords: Angiogenesis; Apoptosis; MDA-MB-231; Vascular endothelial growth factor; VEGF

1. Introduction

Epidemiological studies indicate that diet and nutrition influence the development of breast cancer [1,2]. The highest rate of breast cancer is observed in populations with western lifestyles that include relatively high fat, meat-based, low fiber diets whereas the lowest rates are typically observed in Asian populations with mainly plant-based diets. The high content of phytoestrogens in these plant-based diets has been proposed as the underlying factor responsible for the low breast cancer incidence in Asian women but the mechanisms are relatively unexplored [3]. Resveratrol is a phytoestrogen suggested to have marked chemopreventative properties [4].

Resveratrol's structural similarity to diethylstilbestrol, a synthetic estrogen, has led to investigations into resveratrol's estrogenic activity. Resveratrol has been shown to bind to estrogen receptor alpha (ERα) and activate transcription of estrogen regulated genes [5]. It has also been shown that resveratrol binds a second subtype of estrogen receptor, namely estrogen receptor beta (ERβ), with comparable or slightly greater affinity than that to ERα [6]. However, the selective ER mediated effects of resveratrol remain controversial [6,7], although the differential effects observed in various cell lines may be due to the relative expression

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levels of ERα and ERβ [8]. The effects of resveratrol on breast cancer cell growth appear to be dose-dependent. Low concentrations of resveratrol appear to have a growth-stimulating effect on ERα-positive breast cancer lines [5,9], while in the presence of estradiol, resveratrol has been shown to inhibit estradiol stimulated growth as well as estradiol mediated transcription [9,10]. Moreover, at higher concentrations resveratrol has been shown to inhibit growth of both ERα positive and ERα negative cell lines [9–11]. Thus, similarly to tamoxifen, resveratrol appears to act as a partial estrogen receptor agonist at low concentrations while antagonizing the effects of estrogen at higher concentrations. Resveratrol's growth inhibiting effects on ERα negative cell lines, however, suggest that antagonism of the ERα receptor is not the sole mechanism for growth inhibition in breast cancer cell lines [10,11].

In vivo studies of the estrogenic effects of resveratrol are limited, but the majority of those conducted thus far have not confirmed the ERα agonistic effects suggested by in vitro studies [12,13]. Rather, resveratrol appears to have pure antiestrogenic effects at high doses [12]. In addition, resveratrol has been shown to inhibit the formation of carcinogen-induced preneoplastic mammary lesions and tumors in rodent models [8].

It is widely accepted that the growth of a solid tumor such as breast cancer is dependent on angiogenesis [14,15]. High levels of vascular endothelial growth factor (VEGF), one of the most potent and specific angiogenic factors, have been shown to correlate with poor prognosis and decreased overall survival for both node-positive and node-negative breast cancer patients [16,17]. Estrogen exposure is considered a major risk factor for breast cancer development, and the majority of breast cancers remain hormone dependent [18–20]. In the VEGF gene, an estrogen responsive element (ERE) has been identified [10] and increased VEGF mRNA expression has been found in breast cancer tissue, compared to adjacent normal breast tissue [21]. Both in vitro and in vivo studies have indicated that sex steroids can induce VEGF expression in normal breast tissue and in breast cancer [22–25]. However, very little is known about the effects of phytoestrogens such as resveratrol on VEGF and about the possible role of VEGF regulation of breast cancer growth. One hypothesis is that VEGF, besides mediating angiogenesis, also functions as an autocrine factor on breast carcinoma cells to maintain their survival [26].

The aim of this study was to investigate the effects of resveratrol on ERα negative, ERβ postitive MDA-MB-231 tumor growth, angiogenesis, and VEGF levels. We have chosen to investigate the effects of resveratrol on VEGF levels in the extracellular space, where VEGF is biologically active. Our results suggest that resveratrol decreases extracellular VEGF levels in vitro, inhibits angiogenesis in vivo, and induces apoptosis in MDA-MB-231 cells with significant tumor-reducing effects in vivo.

2. Materials and methods

2.1. Cell culture and experimental conditions

ERα negative ERβ positive MDA-MB-231 human breast adenocarcinoma cells were obtained from ATCC (Manassas, VA, USA) and cultured in Dulbecco's medium without phenol red supplemented with 10% fetal bovine serum, 50 IU/ml penicillin, 50 μg/ml streptomycin, and 1% 2 mM glutamine (all supplements purchased from Life Technologies, Inc., Paisly, UK). The cells were incubated at 37 °C in humidified air with 5% CO_2 and subcultured once a week.

Prior to experiments the cells were trypsinized (0.05 trypsin, 0.02% ethelenediaminetetraacetic acid, EDTA), resuspended in the medium described above, and plated into Petri dishes (Costar, Cambridge, MA, USA) at a density of 15,000 cells/cm^2. After 24 h, the medium was removed and replaced by serum-free medium consisting of equal amounts Dulbecco's medium without phenol red and nutrient mixture F-12 (HAM) (Life Technologies, Inc.) supplemented with 0.2 mg/ml bovine serum albumin, 10 μg/ml transferrin, and 1 μg/ml insulin (all three supplements purchased from Sigma, St Louis, MO, USA). After an additional 24 h, the serum-free medium was removed and replaced with serum-free medium with 100 μM resveratrol prepared from stock solutions (35 mM) dissolved in ethanol. This concentration is comparable to resveratrol's IC_{50} value for ERβ [6,27]. The maximal ethanol concentration never exceeded

0.46%. Controls were given serum-free medium without resveratrol.

2.2. Determination of caspase activity

Caspase activity can be determined using Ac-DEVD-7-amino-4-methyl-coumarine (Ac-DEVD-AMC), a substrate specifically cleaved by caspase 3-like caspases. Cells were collected and the pellets frozen until analysis, which was conducted according to the manufacturer's instructions (Beckton-Dickinson, San Diego, CA, USA). The amount of AMC was measured at $\lambda_{ex}380$ $\lambda_{em}435$ by a spectrofluorometer RF-540 (Shimadzu, Kyoto, Japan). A standard curve was constructed using AMC (Sigma), and the data was expressed as nmol AMC per hour per milligram total cellular protein determined as described by Lowry [28].

2.3. Giemsa staining

Cells were removed from the Petri dishes by scraping, washed in phosphate buffered saline, PBS, and thereafter cyto-centrifuged for 5 min at $10\times g$ in a Shandon cytospin 2 (Shandon, Inc., Pittsburgh, PA, USA). The cells were fixed in 4% neutral buffered formaldehyde for a minimum of 20 min, washed with water, and then stained for 2 min with 5% Giemsa solution (Merck, Darmstadt, Germany). Using a light microscope at 400× magnification, five areas were selected randomly and the number of cells with and without condensed nuclei was counted by a single investigator blinded to the treatment group.

2.4. Flow cytometry of propidium iodide-stained cells

Cells were trypsinized, washed in ice-cold PBS, and the pellet was resuspended immediately prior to analysis in a solution consisting of 200 mg tri-sodium-dihydrate, 200 μl nonidet P40, 336.35 mg spermine tetrahydrochloride, 12.1 mg trizma base, 83.19 mg propodium iodide, and 200 ml distilled water (pH 7.6). DNA content was then analyzed on a flow cytometer (BD LSR, Beckton Dickinson, San Jose, CA, USA). Ten thousand events per sample were analyzed [29].

2.5. Electron microscopy

Following resveratrol treatment, the cells in the Petri dishes were fixed in situ by adding 2% glutaraldehyde (Agar Scientific, Essex, UK) in 0.1 M sucrose–sodium cacodylate–HCl buffer (pH 7.2) and post-fixed in osmium tetroxide (Johnson Matthey Chemicals, Roystone, UK). Dehydration, en bloc staining with uranyl acetate, repeated dehydration, and embedding in Epon-812 (Fluka AG, Buchs, Switzerland) were then performed in the Petri dishes. Thin sections of the cured blocks were cut using a diamond knife and thereafter, stained with lead citrate. The specimens were examined at 80 kV in a JEOL 1200-EX electron microscope and digitally photographed [30].

2.6. Quantification of VEGF

Cell culture media were analyzed for VEGF using a commercial quantitative immunoassay kit for human VEGF (QuantGlo, human VEGF; R&D systems, Abingdon, UK) without preparation. According to the manufacturer, this kit measures the VEGF 165 and 121 isoforms (mean minimal detectable dose 1.76 pg/ml, intra-assay and inter-assay precision 3–8%). The precision of the ELISA kit has been confirmed in our laboratory previously [25]. Protein content was determined using the method described by Lowry [28].

2.7. Animal experiments

Female athymic mice (6–8 weeks old) were purchased from M&B, Ry, Denmark. They were housed in a pathogen-free isolation facility with a light/dark cycle of 12/12 h and fed with rodent chow and water ad libitum. The Linköping University animal ethics research board approved all animal work. MB-MDA-231 cells (5×10^6 cells in 200 μl PBS) were injected s.c. on the right hind flank. Tumor volume was determined by measuring length, width, and depth of the tumor every 5 days using a caliper. At a tumor size of approximately 40 mm^3 the mice were divided into two subgroups. One group received daily intraperitoneal injections of resveratrol dissolved in ethanol at a dose of 25 mg/kg per day while the other group received daily injections of vehicle only.

Following 3 weeks of treatment, the mice were sacrificed and the tumors and livers fixed in 4% paraformaldehyde, sectioned, and stained with hematoxilin–eosine for light microscopic analysis.

2.8. TUNEL staining

Detection of DNA fragmentation was performed according to the manufacturer's instructions using an in situ cell death detection kit (Roche, Mannheim, Germany). Briefly, the sections were permeabilized with 0.01 M citrate buffer (pH 6) and then incubated with TUNEL reaction mixture including enzyme solution and labeling solution (fluorescein-labeled nucleotides) for 1 h at 37 °C. After washing with PBS, the sections were incubated with alkaline phosphatase-conjugated anti-fluorescein antibodies for 30 min at 37 °C. The sections were washed with PBS, and the substrate, Fast Red (Roche), was added followed by counterstaining with hematoxilin. The apoptotic index was calculated as follows: (number of apoptotic nuclei/number of total nuclei) × 100. Three measurements were obtained from the border zone area of each by a single investigator blinded to the treatment group.

2.9. Immunohistochemistry of tumor sections

Formalin-fixed, paraffin embedded tumors were cut in 3 μm sections, deparaffinized and subjected to anti-von Willebrand's factor (rabbit anti-human von Willebrand, dilution 1:1000, DAKO). Sections were counterstained with Mayer's hematoxylin. Negative controls did not show staining. In a blinded manner three hot-spots were examined per tumor section (high power fields × 200) of three different tumors in each group. Vessel quantification of tumor sections was conducted as previously described [31] using a Nikon microscope equipped with a digital camera. Percent of area stained positively for von Willebrand's factor was assessed using easy image measurement software (Bergstrom Instruments).

2.10. Statistical analyses

Kruskal–Wallis and Mann–Whitney tests were applied as appropriate. Differences were considered significant when $P < 0.05$. The reported values represent the median, 25th and 75th percentiles.

3. Results

To explore the effects of resveratrol on apoptosis and tumor growth in vivo, nude mice carrying size-matched MDA-MB-231 tumors were treated daily with 25 mg/kg resveratrol for 3 weeks. Weekly measurement of tumor size revealed a significant inhibition of tumor growth in the resveratrol-treated group. After 3 weeks, tumors in the untreated group were approximately five times larger than their size at start of treatment, while resveratrol-treated tumors showed a decrease in size ($P < 0.05$ and $P < 0.01$ between resveratrol-treated mice and controls at weeks 2 and 3, respectively; Fig. 1; $n = 5$). The median tumor size in the resveratrol-treated group at week 3 was 4 mm^3 (25th, 75th percentiles 0, 54 mm^3) compared to 182 mm^3 in the control group (25th, 75th percentiles 160, 210 mm^3). As shown in Table 1, all five tumors in the control group increased in size, while three of the five tumors in the resveratrol-treated group decreased to such an extent that they were no longer macroscopically detectable. It may be noted that these resveratrol-treated tumors were not available for immunohistochemical analysis. Resveratrol-treated mice showed no weight loss or other signs of

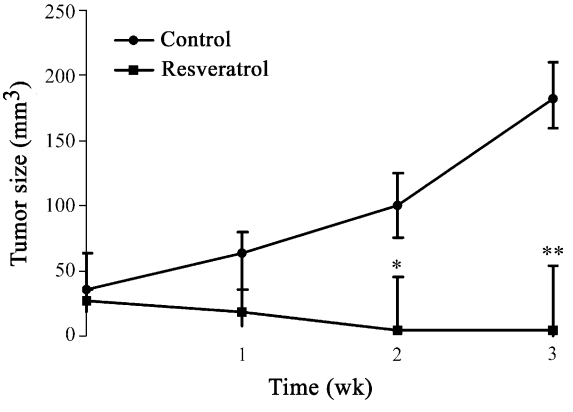

Fig. 1. Effects of resveratrol on MDA-MB-231 tumor size in nude mice treated with intraperitoneal injections of resveratrol (25 mg/kg per day) for 3 weeks. Data represent the median tumor size in mm^3 assessed by caliper measurement ± the 25th and 75th percentiles (*$P < 0.05$ at week 2 and **$P < 0.01$ at week 3).

Table 1
Effects of intraperitoneal injection of resveratrol (25 mg/kg per day) for 3 weeks on MDA-MB-231 tumor size in nude mice

Treatment	No response[a]	Partial response[a]	Total regression[a]
Control	5/5	0/5	0/5
Resveratrol	0/5	2/5	3/5

[a] Partial response denotes decrease in tumor size; total regression denotes tumors that were macroscopically undetectable following treatment. All five tumors in control mice showed continued tumor growth, described here as no response.

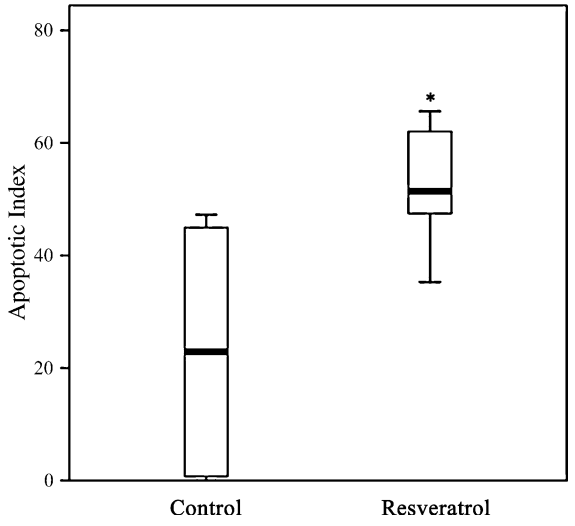

Fig. 2. TUNEL staining of MDA-MB-231 tumor sections from resveratrol-treated mice. The apoptotic index was calculated as described in Section 2 and illustrated in a box plot where the horizontal line in the box represents the median, the upper and lower lines of the box the 75th and 25th percentiles, and the uppermost and lowermost lines the extreme values (*$P<0.05$).

systemic toxicity compared to controls. In addition, liver sections from resveratrol-treated mice showed no evidence of pathology compared with those from controls (data not shown).

TUNEL-staining was performed on tumor sections prepared from resveratrol-treated mice and controls after 3 weeks of treatment. An increase in apoptotic index was observed in resveratrol-treated tumors, $P<0.05$ compared with controls (Fig. 2; $n=2$–3). In addition, significantly decreased vessel area was observed on tumor sections from animals treated with resveratrol, 3.3% (25th, 75th percentiles 3.3, 3.5) vs. 5.5% (25th, 75th percentiles 5.2, 6.0%) in the control group, $P<0.01$ (Fig. 3). The results are in line with our in vitro data, which showed a decreased secretion of VEGF after resveratrol treatment.

A family of cysteine proteases called caspases plays a central role in executing apoptosis [32]. In this study caspase activity was determined using Ac-DEVD-AMC, a substrate specifically cleaved by caspase 3-like caspases. In MDA-MB-231 cells treated with 100 μM resveratrol, the caspase activity increased steadily over a period of 48 h. Cells treated with resveratrol for 48 h exhibited significantly higher caspase activity than control cells [16.1 nmol/mg protein/h (25th, 75th percentiles 11.3, 19.7 nmol/mg protein/h) vs. controls 6.9 nmol/mg protein/h (25th, 75th percentiles 5.7, 9.4 nmol/mg protein/h) $P<0.05$ (Fig. 4; $n=5$–7)].

In addition to the activation of caspases, apoptosis is also characterised by morphological changes including nuclear and cytoplasmic condensation, plasma membrane blebbing, and the building of so-called apoptotic bodies, enclosed vesicles containing cellular content. After initial experiments using 10–100 μM resveratrol, 100 μM was found to induce significant morphological changes indicative of apoptosis. Therefore, this concentration was chosen for all

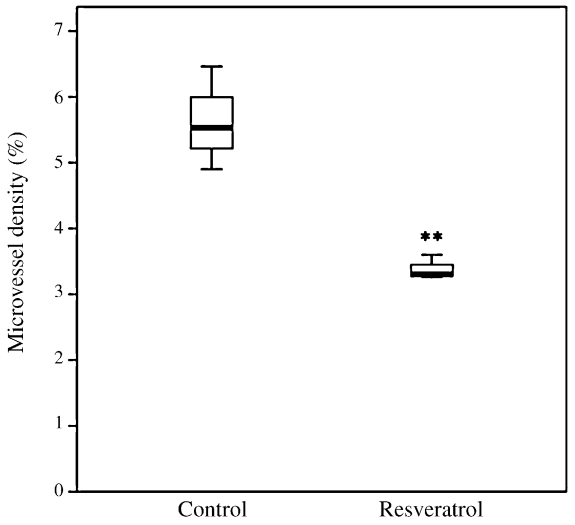

Fig. 3. Microvessel density in MDA-MB-231 tumor sections from resveratrol-treated mice. Mice were treated as described in Fig. 1 and tumor sections were stained with anti-von Willebrand's factor. Vessel area was determined as described in Section 2. Data are presented in a box plot as described in Fig. 2 (**$P<0.01$).

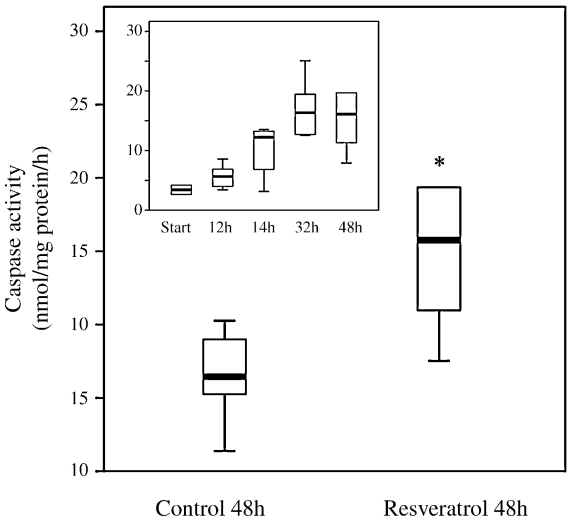

Fig. 4. Caspase activity in MDA-MB-231 cells in culture following incubation with serum-free medium alone or with 100 μM resveratrol for 48 h (*$P<0.05$). Inset illustrates caspase activity in MDA-MB-231 cells incubated with 100 μM resveratrol for 0–48 h. See Fig. 2 for clarification of the box plot.

in vitro experiments. In our study we found that the percentage of apoptotic cells as assessed by Giemsa staining increased steadily in resveratrol-treated MDA-MB-231 cells over a period of 48 h. A significant increase in apoptotic cells was observed after 48 h [38% apoptotic cells (25th, 75th percentiles 33, 41%) vs. controls 6% apoptotic cells (25th, 75th percentiles 2, 11%) $P<0.05$ (Fig. 5; $n=3$–6)]. Analysis of propidium iodide-stained cells revealed a similar increase in apoptotic cells after 48 h of resveratrol treatment, with approximately two and a half times as many cells with fragmented DNA as time-matched controls (Fig. 6; $P<0.01$; $n=5$). Apoptotic cell death was confirmed by transmission electron microscopy. Changes in nuclear morphology typical of apoptosis, including marginal condensation of chromatin, could be seen in cells treated with resveratrol for 24 h. Large cytoplasmic vacuoles were also observed. Similar results were observed for cells incubated with resveratrol for 48 h. Control cells showed intact cytoplasmic organelles, evenly distributed chromatin, and well-preserved nuclear membranes (electron microscopy not shown). Control cells incubated with 0.46% ethanol vehicle showed morphological characteristics similar to those incubated in serum-free medium alone.

In order to investigate the effects of resveratrol on the potent angiogenic stimulator VEGF, we determined the VEGF levels in culture media using a quantitative ELISA. VEGF levels in medium of MDA-MB-231 cells treated with 100 μM resveratrol for 48 h were approximately half of VEGF levels in medium from controls, incubated in hormone medium alone for 48 h [3.9 ng/mg protein (25th, 75th percentiles 3.9, 4.2 ng/mg) vs. controls 8.9 ng/mg (25th, 75th percentiles 7.5, 8.9 ng/mg) $P<0.05$]. VEGF is secreted from viable cells, while caspases become active in cells undergoing apoptosis. This together with non-synchronized cell death, may explain why the caspase activity leveled off at 32 h and the VEGF levels at 24 h. Results from quantification of VEGF in MDA-MB-231 medium are illustrated in Fig. 7 ($n=3$).

4. Discussion

In this study we have shown that treatment with pharmacological levels of resveratrol inhibited growth of MDA-MB-231 tumor explants in nude mice and that the sections of resveratrol-treated

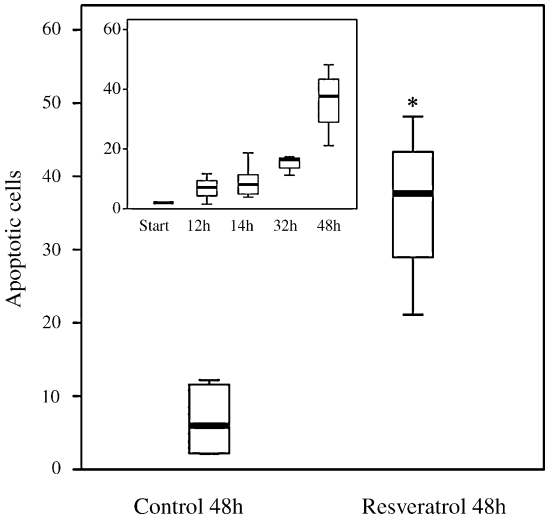

Fig. 5. Quantification of apoptosis induced in MDA-MB-231 cells in culture by 48 h of treatment with 100 μM resveratrol as assessed by Giemsa staining (*$P<0.05$). Inset represents the percentage of cells displaying apoptotic morphology after 0–48 h of 100 μM resveratrol treatment calculated as described in Section 2. Data are presented in a box plot as described in Fig. 2.

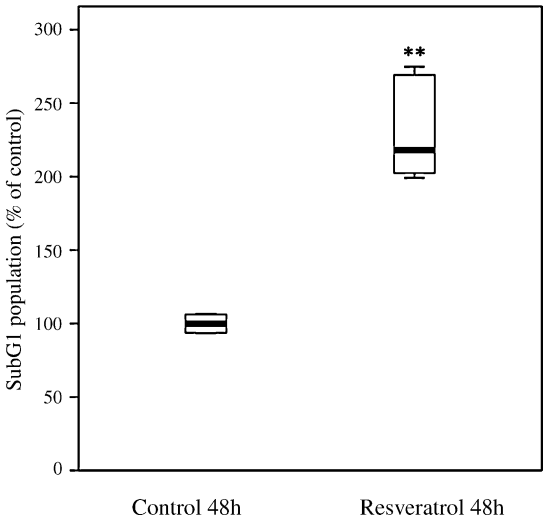

Fig. 6. Flow cytometry of propidium iodide-stained MDA-MB-231 cells following 48 h of treatment with 100 μM resveratrol compared to time-matched controls (**$P<0.01$). The SubG1 population for each treatment group is presented in a box plot as clarified in Fig. 2.

tumors exhibited increased apoptosis and decreased angiogenesis compared with tumors from mice in the control group. In vitro, MDA-MB-231 cells exposed to resveratrol showed an increase in caspase activity as well as induction of morphologic changes characteristic of apoptosis illustrated by electron microscopy and measured quantitatively by Giemsa staining and flow cytometry. Moreover, resveratrol decreased the secretion of VEGF into cell culture media in vitro.

Several modes of action have been proposed and studied regarding the beneficial effects of resveratrol on breast cancer growth. Resveratrol is able to mimic the activity and effects of endogenous estradiol, which would seem to contradict the beneficial effects of resveratrol on the breast. However, it has been shown that resveratrol acts as a mixed agonist/antagonist on the ER and exhibits higher transcriptional activity when bound to ERβ than to ERα [6]. Moreover, resveratrol seems to have antagonist activity with ERα but not with ERβ [6]. MDA-MB-231 is an ERα negative ERβ positive human breast adenocarcinoma cell line [33–35], thus the growth-inhibiting effects of resveratrol on MDA-MB-231 may be mediated by ERβ or may be ER-independent. The role of ERβ in the human breast is not well understood [36] and confounding factors such as ovarian dysfunction and ERβ splice variants in ERβ−/− mice makes interpretation of results from murine models difficult [37]. Nonetheless, it has been reported that 17β-estradiol as well as tamoxifen, 4-hydroxytamoxifen, and raloxifene can induce apoptosis by an ERα-independent pathway that is caspase-dependent [38]. Moreover, other in vitro studies suggest that ERβ may indeed mediate apoptosis [39]. Resveratrol as well as estrogen have been shown to modulate the cell cycle via various other pathways. One example of the non-genomic effects of resveratrol is the inhibition of MAP kinase phosphorylation [40]. Previous studies have also suggested that resveratrol inhibits ribonucleotide reductase in MDA-MB-231, thus regulating the availability of the precursors of DNA synthesis [41]. In addition, resveratrol inhibits NADH ubiquinone oxidoreductase and DNA polymerase, providing additional explanations for how it could modulate the cell cycle [42,43]. A study in MCF-7 breast cancer cells has revealed a novel ER-independent pro-apoptotic mechanism for resveratrol involving the activation of the cAMP/kinase-A system [44]. Non-apoptotic cell death of MDA-MB-231 cells after resveratrol treatment has been observed in a previous study [41], which is in disagreement with our present results. This discrepancy may be explained by

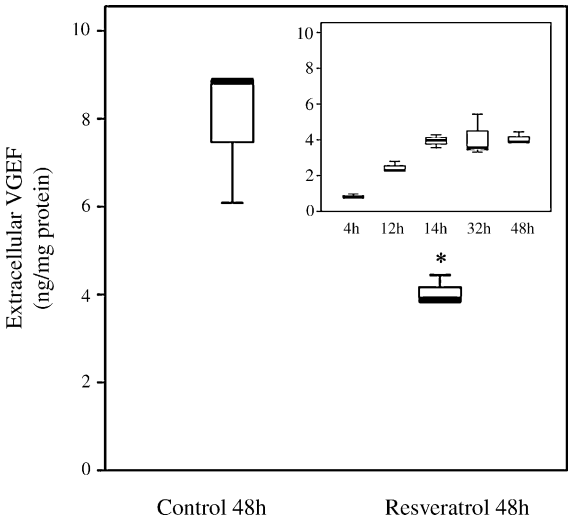

Fig. 7. Extracellular VEGF in MDA-MB-231 cell culture media after 48 h of 100 μM resveratrol treatment (*$P<0.05$). Inset shows VEGF levels in culture media of cells treated with 100 μM resveratrol for 4–48 h. VEGF levels were measured using ELISA. See Fig. 2 for clarification of the box plot.

differences in methodology, both of cell culturing and measuring apoptosis. In our present study we have applied several techniques for determining apoptosis, both enzymatic and morphological methods, namely caspase activity, flow cytometry of propodium iodide-stained cells, Giemsa-staining, and electron microscopy. All these different techniques verified apoptosis in MDA-MB-231 cells after resveratrol exposure.

In the present study we have also shown that resveratrol significantly decreased the levels of extracellular VEGF secreted from MDA-MB-231 cells. Using microdialysis, we have previously illustrated similar reductions in extracellular VEGF in another human breast cancer model after diet supplements of phytoestrogen in flaxseed, a rich source of lignans [45]. In this study, due to the lack of palpable tumors in the resveratrol-treated group, microdialysis for in vivo measurement of tumor-secreted VEGF was not possible. However, we show with immunohistochemistry decreased angiogenesis in resveratrol-treated tumors. It has previously been reported that resveratrol may inhibit angiogenesis by suppressing the action of VEGF on endothelial cells by reducing the phosphorylation of MAP kinase and thus blocking the VEGF receptor mediated response [40]. This taken together with our present results suggests that resveratrol may affect angiogenesis at different levels, both by reducing the secretion of VEGF and by influencing downstream signaling pathways of the VEGF receptor. Treatment with estradiol has also been shown to reduce VEGF levels in MDA-MB-231 cells [46], suggesting the resveratrol's effects on VEGF may be mediated by ERβ. VEGF is considered to play an essential role in breast cancer progression by stimulating angiogenesis [47]. In addition, Bachelder and co-workers have proposed that VEGF functions in an autocrine fashion, stimulating signaling pathways mediating cell survival [26]. Thus, the inhibition of tumor growth observed in vivo may indeed be the synergistic result of resveratrol's apoptosis-inducing and anti-angiogenic effects.

Breast cancer is the most common cancer and a major cause of death among women in the Western world today. Selective estrogen receptor modulators (SERMs) are cornerstones in breast cancer treatment and have also shown to have promising effects on reducing the incidence of breast cancer in women at high risk of developing this disease. However, the most widely used SERM, tamoxifen, may also promote the development of endometrial cancer. Resveratrol has been characterized to function as a SERM [8] and there is evidence that resveratrol is not likely to facilitate endometrial carcinogenesis [12,13] and may indeed confer other health benefits such as cardioprotection [48].

The results of our study are in agreement with previous reports from other investigators, suggesting a chemotherapeutic potential of resveratrol. To our knowledge we are the first to illustrate the growth-inhibiting effects of resveratrol on established human mammary tumors in vivo in nude mice. We speculate that the tumor-reducing effects of resveratrol may be due to synergism between the induction of apoptosis and the inhibition of angiogenesis through VEGF. These effects may, however, be dependent on the initial tumor size, as unpublished observations from our laboratory suggest a limited effect of resveratrol on larger tumors. It can be argued that the dose of resveratrol used in this study was at a pharmacological level which cannot be achieved by a normal food and drink intake. Nonetheless, we could not detect any toxic effect of resveratrol on the animals. There were no differences in well-being or behavior of the mice and examinations of liver sections showed no toxic effects after resveratrol treatment compared with controls. Our results suggest that resveratrol has a potent effect on breast cancer tumor growth and may prove to have a role in breast cancer treatment/prevention in the future. Further studies are warranted to determine the chemotherapeutic and chemopreventive effects of resveratrol on human breast cancer.

Acknowledgements

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