Effect of resveratrol on the metastasis of 4T1 mouse breast cancer cells in vitro and in vivo

Hyun Sook Lee 1, Ae Wha Ha 2 and Woo Kyoung Kim 2
1 Department of Food Science and Nutrition, Dongseo University, Busan 617-716, Korea
2 Department of Food Science and Nutrition, Dankook University, 126, Jakjeon-dong, Suji-gu, Yongin-si, Gyunggi 448-701, Korea

Abstract
We investigated the effects of resveratrol on metastasis in in vitro and in vivo systems. 4T1 cells were cultured in the presence of various concentrations (0–30 μmol/L) of resveratrol. For experimental metastasis, BALB/c mice were injected intravenously with 4T1 cells in the tail vein, and were orally administered various concentrations (0, 100, or 200 mg/kg Body weight) of resveratrol for 21 days. After resveratrol treatment, cell adhesion, wound migration, invasion, and MMP-9 activity were significantly decreased in a dose-dependent manner in 4T1 cells (P < 0.05). The numbers of pulmonary nodules were significantly decreased in mice fed the resveratrol (P < 0.05). The plasma MMP-9 activity was decreased in response to treatment with resveratrol in mice (P < 0.05). We conclude that resveratrol inhibits cancer metastasis both in vitro and in vivo, and this inhibition is likely due to the decrease in MMP-9 activity caused by resveratrol.

Key Words: Resveratrol, metastasis, matrix metalloproteinase-9, 4T1 breast cancer cell, BALB/c mice

Introduction
Resveratrol (trans-3',4',5'-trihydroxystilbene) is a naturally occurring polyphenolic phytoalexin that is present in abundance in many fruits, such as grapes, as well as in wine [1]. Resveratrol intake has been reported to have anti-inflammatory and anti-atherosclerosis function and to modulate hepatic lipoprotein and lipid synthesis, platelet aggregation, and production of antiatherogenic eicosanoids by human platelets and neutrophils [2-7]. Therefore, red wine polyphenol resveratrol is believed to be responsible for the well known phenomenon of the French Paradox [8-9]. In addition, resveratrol has recently been found to exhibit anti-cancer properties. Specifically, resveratrol has been found to inhibit the proliferation of a wide variety of tumor cells including breast, lung, colon, liver, pancreas, skin, and prostate cancer cells [10,11]. However, the precise mechanisms by which the anticarcinogenic effects of resveratrol occur remain largely unknown.

Cancer metastasis consists of a complex cascade of events, which ultimately allow for tumor cell escape and seeding of ectopic environments [12]. For breast cancer cells to manifest their malignant potential, they must develop the ability to break through and dissolve extracellular matrix (ECM), particularly the delimiting basement membrane (BM). The degradation of the pericellular BM and ECM is catalyzed by the concerted action of several classes of ECM-degrading enzymes. One important class of ECM-degrading enzymes is the matrix metalloproteinases (MMPs) [13]. MMPs have been implicated as possible mediators of invasion and metastasis in some cancers. Among the human MMPs, gelatinase-B (MMP-9) is the key enzyme that degrades type IV collagen [14]. MMP-9 is overexpressed in invasive tumor and thus it may play an important role in cancer invasion through its enzymatic degradation of the extracellular matrix [15,16].

The purpose of this study is evaluating the effects of resveratrol on the metastasis of the 4T1 mouse breast cancer cell line derived from a spontaneously arising BALB/c mammary tumor, closely resembles breast cancer in humans [17], both in vitro and in vivo.

Materials and Methods

Chemicals and reagents
Resveratrol was purchased from Sigma (Sigma R5010, St Louis, MO, USA), dissolved in dimethyl sulfoxide (DMSO, Sigma D2650) and then diluted in cell culture medium. The mouse breast cancer cell line, 4T1, was purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The 4T1 mouse mammary tumor cell line is one of breast cancer models with the capacity to metastasize efficiently to sites...
affected in human breast cancer [17]. The following reagents and chemicals were also utilized in this study: Dulbecco's modified Eagle's medium/Ham's F12 Nutrient Mixture (DMEM/F12), streptomycin, and penicillin (Gibco/BRL, Grand Island, NY, USA); and RIA-grade bovine serum albumin and transferrin (Sigma). In addition, antibodies for MMPs were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). All other reagents were obtained from Sigma (St. Louis, MO, USA).

Cell culture

4T1 cells were maintained in DMEM/F12 medium containing 100 mL/L of fetal bovine serum (FBS), 100,000 U/L of penicillin and 100 mg/L of streptomycin. To examine the effects of resveratrol, 4T1 cells were plated with DMEM/F12 medium containing 10% FBS. Before the cells were treated with resveratrol, the cell monolayers were rinsed and then serum starved for 24 h in DMEM/F12 medium supplemented with 5 mg/L of transferrin, 0.1 g/L of BSA, and 5 μg/L of selenium. After serum starvation, the cells were supplied with fresh serum-free medium (SFM) containing the indicated concentrations of resveratrol.

Adhesion assay

The adhesion assay was performed as described previously [9]. Briefly, 96-well plates were coated with fibronectin at a concentration of 0.2 g/L phosphate buffered saline (PBS) (BD Biosciences, MA, USA,) and then incubated at 37°C for 1 h under 5% CO₂. 4T1 cells (8 × 10⁵ cells/ well) were suspended in medium containing 0, 10, 20, or 30 μmol/L resveratrol were seeded into the coated wells. The samples were then incubated for 1 h at 37°C, after which the adherent cells were washed with PBS and reincubated in medium containing 1 g/L of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) for 3 h at 37°C. The filters were then stained with Diff-Quik stain solution (Dade Behring, Newark, NJ, USA), after which the cells on the lower surface of the filter were fixed onto a glass slide. Finally, cells in five randomly selected microscopic fields (x400) of the lower slide were then counted.

MMP-9 activity (gelatin zymography)

MMP-9 activity was investigated as described previously [18]. Cells were seeded into a 6-well plate at a concentration of 1 × 10⁶ cells/mL. The monolayers were then incubated in serum-free medium containing 0, 10, 20, and 30 μmol/L resveratrol for 16 h. The supernatants were then collected and concentrated using Centricon centrifugal filter devices (Millipore). The supernatant was mixed with 2x sample buffer (Invitrogen), after which zymography was performed using gels (10% polyacrylamide, 1% gelatin). The MMP activity was then visualized by staining the gels with Coomassie blue.

MMP-9 mRNA expressions

Reverse transcriptase polymerase chain reaction was performed as previously described [19]. Total RNA was isolated using TRIzol reagent (Sigma), after which cDNA was synthesized using 2 μg of the isolated total RNA and SuperScriptTM II reverse transcriptase (Invitrogen). Next, primers for MMP-9 (upstream primer, 5'-TGGGCTACGTGACCTATGACCAT-3', downstream primer, 5'-GCCCAAGCCACCCACTCCACTC-3'), and annealed at 55°C for 1 min for 35 cycles were used to amplify the cDNA.

Animal and diet

Forty female BALB/c mice (Central Lab. Animal Inc., Korea) that were approximately 5 weeks of age, 15-20 g in weight and housed in groups of 5 were used in this study. The animals were divided into four treatment groups (10/group), namely controls (Con), tumor hosts (Can), tumor hosts + 100 mg/kg BW resveratrol (CanR100), and tumor hosts + 200 mg/kg BW resveratrol (CanR200).
resveratrol (CanR200). The mice were fed an AIN-96G control diet [20] until the experiments commenced. The food intake was measured twice a week and the weight of the mice was measured daily. Animals were maintained at 22 ± 2°C on a regular light-dark cycle and provided with free access to food and water. All animal studies were conducted in accordance with the Dankook University ethics committee’s guidelines for the care and use of laboratory animals.

### Tumor inoculation and treatment

The tumor hosts received inoculums of $2 \times 10^5$ 4T1 cells intravenously. The CanR100 and CanR200 groups were then orally administered a daily dose of resveratrol dissolved in a 2% ethanol at doses of 100 and 200 mg/kg BW, respectively. The Con and Can groups were administered only the 2% alcohol solution. On day 21, the animals were weighed and then anesthetized with ethyl ether, after which they were sacrificed and their blood collected by heart puncture. The plasma was separated and stored at -80°C until the subsequent analysis of MMP-9 activity. In addition, the lungs were collected after the blood was removed and then visually examined for metastasis. The number of metastases was then determined by staining with Bouin’s solution (saturated picric acid/formalin:acetic acid = 15:5:1) after washing the lungs with PBS. The MMP-9 activity in plasma was investigated using gelatin zymography, which was performed using gels (10% polyacrylamide, 1% gelatin). MMP-9 activity was visualized by staining with Coomassie blue.

### Statistical analysis

Statistical analysis was performed using the Statistical Analysis System (SAS Institute, Cary, NC). Data were expressed as the means ± standard deviations and then compared among groups by analysis of variance (ANOVA). Statistically significant differences among groups were further tested at $\alpha = 0.05$ using Duncan’s multiple range test.

### Results

#### Effects of resveratrol on the adhesion, motility, and invasion in 4T1 cells

As shown in Fig. 1A, resveratrol decreased the adhesion of 4T1 cells in a dose-dependent manner. Treatment of 4T1 cells

![Fig. 1. The effects of resveratrol on the adhesion, motility, and invasion in 4T1 cells.](image)

(A) After $8 \times 10^5$ cells/mL suspended in DMEM/F12 medium containing 0, 10, 20, or 30 μmol/L resveratrol were plated in each well of a 96 well fibronectin-coated plate for 1 h, the medium was gently removed and the attached cells were subjected to an MTT assay. (B) Cells were plated in a 12-well plate at a density $5 \times 10^5$ cells/well in DMEM/F12 supplemented with 10% FBS. Confluent monolayers were then wounded and subsequently incubated in serum free medium in the presence of 0, 10, 20, or 30 μmol/L resveratrol. The cells were then photographed under a phase contrast microscope at 0, 12, 24, and 48 h after being wounded. (C) Cells were cultured in the presence of various concentrations of resveratrol for 8 h in a Boyden chamber. a) Microphotography of cells treated with resveratrol, b) Quantitative analysis of the cell motility assay. (C) Cells were cultured in the presence of various concentrations of resveratrol for 8 h in an invasion chamber a) Microphotography of cells treated with resveratrol, b) Quantitative analysis of the invasion assay. Each bar represents the mean ± SD of three independent experiments. Significant differences ($P < 0.05$) among groups are indicated by different letters above each bar.
Effects of resveratrol on the activity and mRNA expression of MMP-9 in 4T1 cells

The activity of MMP-9 in 4T1 cells was decreased in response to treatment with resveratrol in a dose-dependent manner (Fig. 2A). Furthermore, the results of RT-PCR analysis suggested that the expression of MMP-9 mRNA was decreased in 4T1 cells in response to treatment with resveratrol (Fig. 2B). These results suggested that resveratrol could play a crucial role in the down-regulation of MMP-9 expression.

Effects of resveratrol on lung metastasis in vivo

We examined the effect of resveratrol on the metastasis of 4T1 cells in BALB/c mice. Tumor burden and the administration of resveratrol had no effects on food intake. However, tumor burden caused a significant reduction in body weight, irrespective of treatment with resveratrol (Table 1). The macroscopic appearance of the lungs from untreated and treated mice clearly showed that treatment with 100 or 200 mg/kg BW resveratrol reduced the number of 4T1 colonies in the lungs of Balb/c mice (Fig. 3A). With no resveratrol, there were 42.6 ± 11.45 colonies ($P < 0.05$). But there were only 29.3 ± 6.09 with 100 mg/kg and 15.2 ± 8.34 with 200 mg/kg resveratrol. As shown in Fig. 3B, plasma MMP-9 activity was decreased in mice that were treated with resveratrol ($P < 0.05$).
Table 1. The effects of resveratrol on body weight and food intake

| Group          | Initial body weight (g) | Final body weight (g) | Food intake (g/day) |
|----------------|-------------------------|-----------------------|---------------------|
| Con            | 16.8 ± 0.56              | 19.7 ± 0.69           | 1.93 ± 0.18         |
| Can            | 17.0 ± 0.46              | 16.3 ± 1.24           | 2.10 ± 0.12         |
| CanR100        | 17.2 ± 0.51              | 15.7 ± 1.67           | 2.08 ± 0.18         |
| CanR200        | 17.2 ± 0.47              | 16.8 ± 0.95           | 1.98 ± 0.19         |

1) Con, control; Can, tumor inoculation; CanR100, tumor inoculation and treated daily with oral supplementation of 100 mg/kg resveratrol; CanR200, tumor inoculation and treated daily with oral supplementation of 200 mg/kg resveratrol. Each group contained six animals.

2) Mean ± SD
3) NS, not significant
4) Different letters within a column represent a significant difference at α = 0.05 as determined by Duncan’s multiple range test.

Discussion

In this study, the effects of resveratrol on metastasis in 4T1 cells and on experimental metastasis of 4T1 cells in Balb/c mice were evaluated. Cancer metastasis is a complex multi-step process that ultimately enables the escape of tumor cells and the subsequent seeding of ectopic environments [12]. For breast cancer cells to manifest their malignant potential, they must develop the ability to break through and dissolve the ECM, particularly those delineating the BM. The initial invasive action of metastatic cells involves interactions between tumor cells and the ECM that occur via the process of cell matrix adhesion. Once malignant cells have detached from the primary tumor, they bombard the surrounding BMs and adhere to their meshwork of Type IV collagen, laminin, and fibronectin [21]. As shown in Fig. 1A, treatment with resveratrol inhibited the attachment of 4T1 cells to fibronectin, which is one of the major components of the BM.

The process of tumor cell invasion and metastasis requires degradation of the connective tissue associated with vascular BM and interstitial connective tissue [22,23]. The BM is the largest barrier between a free malignant cell and the bloodstream, and it must be traversed before malignant cells can enter circulating blood [24]. Therefore, invasion through a BM is a critical step in metastasis [25]. To successfully penetrate the filter membrane, cells must successfully adhere, degrade, and traverse the Matrigel-coated insert. The present study demonstrated that resveratrol inhibited the invasiveness of 4T1 breast cancer cells in a dose-dependent manner.

Motility is another property of cancer cells that is required for migration from the primary site to a secondary organ to occur. The results presented here demonstrate that treatment with various concentrations of resveratrol reduced the motility of 4T1 cells.

Degradation of the pericellular BM and ECM is catalyzed by the concerted action of several classes of ECM-degrading enzymes. One important class of ECM-degrading enzymes is the MMPs [13]. MMPs have been implicated as possible mediators of invasion and metastasis in some cancers. MMP-9 is a MMP family enzyme that is related to tumor invasion and metastasis due to its capacity for tissue remodeling via the ECM and its ability to degrade the BM [26,27]. In addition, aberrant overexpression of MMP-9 has been found to be associated with increased cancer invasive potential in breast cancer cells [28-30]. Previous reports have shown that resveratrol prevents tumor progression through the inhibition of MMP-9 expression [16,19,31,32] and that it suppresses proinflammatory mediators, such as TNF-α, IL-1β, COX-2, and iNOS [33-35]. The results of the present study demonstrated that resveratrol can inhibit MMP-9 activity and the levels of MMP-9 mRNA in 4T1 cells.

Interestingly, the results of the present study demonstrated that oral administration of resveratrol 21 days either inhibited the growth of 4T1 cells or prevented their metastasis to the lungs in mice. Bove et al. [36] reported that, while it inhibited 4T1 cell proliferation in a dose-dependent manner in vitro, intraperitoneal injection of resveratrol at doses of 1, 3, or 5 mg/kg BW had no effect on in vivo metastasis of 4T1 cells in mice. Conversely, Carbó et al. [37] and Kimura and Okuda [38] reported that treatment with 1 mg/kg and 2.5-10 mg/kg BW resveratrol induced significant decreases in the occurrence of tumor cells and metastasis to the lungs by hepatoma tumor cells and Lewis lung carcinoma cells, respectively. Weng et al. [39] reported that resveratrol suppressed the pulmonary metastasis of BALB/c mice challenged with CT26 colon cancer cells. Furthermore, Busquets et al. [40] demonstrated that the administration of resveratrol had no effect on the growth of an intramuscularly implanted experimental tumor in C57Bl/6 mice. Taken together, the results of these previous studies indicate that the effects of resveratrol on primary tumor growth may be dependent on the experimental model and doses used.

Several resveratrol studies treating animals, the dose is very various from 100 μg to 1000 mg per day [31,41-45]. Comparing with these studies, the concentration of resveratrol of our study is similar to that of Bhat et al. [41], Li et al. [42], Chen et al. [43], and Tseng et al. [44] studies and it is 100 to 200 times higher than that of Banerjee et al. [31] and Harper et al. [45]. Of course, there is a difference on type of treatment that they injected intraperitoneal or subcutaneous, and we supplied orally tube feeding. In our study, there are limitations that we did not set the control group to which we supplied resveratrol without intraperitoneal injection. Our study did not investigate the probability of side effects except food intake and weight induced by high dose of resveratrol. Resveratrol toxicity has been reported at concentration above 10 μmol/L in 549 cells [46]. But other studies showed that had no significant cytotoxic effect of resveratrol was found at a concentration below 100 μmol/L [47]. The deviation between the above results may be related to difference of cell and other experimental conditions or different cytotoxic analysis methods. Therefore, further researches are needed to know whether it would be possible to test the high doses of resveratrol not only on animal but also on human.

This study is the first to report that oral administration of resveratrol inhibits the metastasis of 4T1 cells to the lungs in...
a BALB/c murine model of experimentally induced cancer. Based on these findings, we confirmed that resveratrol may inhibit metastasis by decreasing the activity and expression of MMP-9.

References

1. Zhou HB, Yan Y, Sun YN, Zhu JR. Resveratrol induces apoptosis in human esophageal carcinoma cells. World J Gastroenterol 2003;9:408-11.
2. Kopp P. Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'? Eur J Endocrinol 1998;138:619-20.
3. Jang M, Pezzuto JM. Cancer chemopreventive activity of resveratrol. Drugs Exp Clin Res 1999;25:65-77.
4. Jang M, Cai L, Udeani GO, Slowking KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997;275:218-20.
5. Frankel EN, Waterhouse AL, Kinsella JE. Inhibition of human LDL oxidation by resveratrol. Lancet 1993;341:1103-4.
6. Fauconneau B, Waffo-Teguo P, Huguet F, Barrier L, Decendit A, Merillon JM. Comparative study of radical scavenger and antioxidant properties of phenolic compounds from Vitis vinifera cell cultures using in vitro tests. Life Sci 1997;61:2103-10.
7. Pace-Asciak CR, Rounova O, Hahn SE, Diamandis EP, Goldberg DM. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. Clin Chim Acta 1996;246:163-82.
8. Liu BL, Zhang X, Zhang W, Zhen HN. New enlightenment of French Paradox: resveratrol's potential for cancer chemoprevention and anti-cancer therapy. Cancer Biol Ther 2007;6:1833-6.
9. Sun AY, Simonyi A, Sun GY. The "French Paradox" and beyond: neuroprotective effects of polyphenols. Free Radic Biol Med 2002;32:314-8.
10. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia R, Pezzuto JM. Cancer chemopreventive activity of resveratrol. Drugs Exp Clin Res 1999;25:65-77.
11. Athar M, Back JH, Tang X, Kim KH, Kopelovich L, Bickers DR, Kim AL. Resveratrol: a review of preclinical studies for human cancer prevention. Toxicol Appl Pharmacol 2007;224:274-83.
12. Yoon SO, Kim MM, Chung AS. Inhibitory effect of selenite on inhibition of matrix metalloproteinases in colorectal cancer cells in vitro and in vivo. J Nutr Biochem 2007;18:650-7.
13. Yoon SO, Kim MM, Chung AS. Inhibitory effect of selenite on inhibition of matrix metalloproteinases in colorectal cancer cells in vitro and in vivo. J Nutr Biochem 2007;18:650-7.
14. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939-51.
15. Pignatelli M, Stamp G. Integrins in tumour development and spread. Cancer Surv 1995;24:113-27.
16. Stettler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor interactions with the extracellular matrix during invasion and metastasis. Ann Rev Cell Biol 1993;9:541-73.
17. Soel SM, Choi OS, Bang MH, Yoon Park JH, Kim WK. Influence of conjugated linoleic acid isomers on the metastasis of colon cancer cells in vitro and in vivo. J Nutr Biochem 2007;18:650-7.
18. Schwarzenbacher N, Bovenkamp T, Liotta LA. Matrix metalloproteinases and tumor progression. Nat Rev Cancer 2002;2:161-74.
19. Yang Z, Li, Zeta RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol. Drugs Exp Clin Res 1999;25:65-77.
20. Mook OR, Frederiks WM, Van Noorden CJ. The role of gelatinases in cancer progression. Biochim Biophys Acta 1984;43:533-46.
21. Stermitz FD, Glimm R, Liu Y, Duh EY, El Naggar AK. Pterostilbene inhibited tumor invasion via suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells. Carcinogenesis 2009;30:1234-42.
22. Banerjee S, Bueso-Ramos C, Aggarwal BB. Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloprotease 9. Cancer Res 2002;62:4945-54.
23. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 2007;7:41-51.
24. Manna SK, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappaB, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. J Immunol 2000;164:6509-19.
25. Birrell MA, McCluskie K, Wong S, Donnelly LE, Barnes PJ, Belvisi MG. Resveratrol, an extract of red wine, inhibits lipopolysaccharide induced airway neutrophilia and inflammatory mediators through an NF-kappaB-independent mechanism. FASEB J 2005;19:840-1.
35. Kowalski J, Samojedny A, Paul M, Pietsz G, Wilczok T. Effect of apigenin, kaempferol and resveratrol on the expression of interleukin-1beta and tumor necrosis factor-alpha genes in J774.2 macrophages. Pharmacol Rep 2005;57:390-4.

36. Bove K, Lincoln DW, Tsan MF. Effect of resveratrol on growth of 4T1 breast cancer cells in vitro and in vivo. Biochem Biophys Res Commun 2002;291:1001-5.

37. Carbó N, Costelli P, Baccino FM, López-Soriano FJ, Argilés JM. Resveratrol, a natural product present in wine, decreases tumour growth in a rat tumour model. Biochem Biophys Res Commun 1999;254:739-43.

38. Kimura Y, Okuda H. Resveratrol isolated from Polygonum cuspidatum root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice. J Nutr 2001;131:1844-9.

39. Weng YL, Liao HF, Li AF, Chang JC, Chiou RY. Oral administration of resveratrol in suppression of pulmonary metastasis of BALB/c mice challenged with CT26 colorectal adenocarcinoma cells. Mol Nutr Food Res 2010;54:259-67.

40. Busquets S, Ametller E, Fuster G, Olivan M, Raab V, Argilés JM, López-Soriano FJ. Resveratrol, a natural diphenol, reduces metastatic growth in an experimental cancer model. Cancer Lett 2007;245:144-8.

41. Bhat KP, Lantvit D, Christov K, Mehta RG, Moon RC, Pezzuto JM. Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. Cancer Res 2001;61:7456-63.

42. Li D, Chen X, Yu H. Resveratrol inhibits MMP-2 expression of hepatoma in nude mice. J Anim Vet Adv 2011;10:33-7.

43. Chen Y, Tseng SH, Lai HS, Chen WJ. Resveratrol-induced cellular apoptosis and cell cycle arrest in neuroblastoma cells and antitumor effects on neuroblastoma in mice. Surgery 2004;136:57-66.

44. Tseng SH, Lin SM, Chen JC, Su YH, Huang HY, Chen CK, Lin PY, Chen Y. Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. Clin Cancer Res 2004;10:2190-202.

45. Harper CE, Patel BB, Wang J, Arabshahi A, Eltoum IA, Lamar-tiniere CA. Resveratrol suppresses prostate cancer progression in transgenic mice. Carcinogenesis 2007;28:1946-53.

46. Weng CJ, Yang YT, Ho CT, Yen GC. Mechanisms of apoptotic effects induced by resveratrol, dibenzoylmethane, and their analogues on human lung carcinoma cells. J Agric Food Chem 2009;57:5235-43.

47. Liu PL, Tsai JR, Charles AL, Hwang JJ, Chou SH, Ping YH, Lin FY, Chen YL, Hung CY, Chen WC, Chen YH, Chong IW. Resveratrol inhibits human lung adenocarcinoma cell metastasis by suppressing heme oxygenase 1-mediated nuclear factor-kappaB pathway and subsequently downregulating expression of matrix metalloproteinases. Mol Nutr Food Res 2010;54 Suppl 2:S196-204.