

Inhibitory effect of resveratrol on the expression of the VEGF gene and proliferation in renal cancer cells

RUI YANG¹, HUIZHEN ZHANG² and LIHONG ZHU³

¹Department of Life Science, Xinxiang Medical University, Xinxiang, Henan 453003; ²College of Public Health, Zhengzhou University, Henan 450001; ³Department of Gynaecology and Obstetrics, the First Affiliated Hospital of Xinxiang Medical University, Xinxiang 453100, P.R. China

Received March 21, 2011; Accepted June 10, 2011

DOI: 10.3892/mmr.2011.511

Abstract. Resveratrol (Res) is known to have potent anti-inflammatory and antioxidant effects and to inhibit platelet aggregation and the growth of a variety of cancer cells. In this study, we investigated the effects of Res on the expression of the vascular endothelial growth factor (VEGF) gene and on cell proliferation in human renal cancer (786-0) cells. The 786-0 cells were treated with various concentrations of Res (0, 10, 20 and 40 μ mol/l) for different time periods (24, 48 and 72 h). Cell proliferation was examined by MTT analysis and the expression of the VEGF gene was analyzed RT-PCR and Western blot analysis. Res inhibited the expression of the VEGF gene, and its inhibitory effect increased according to the concentration and treatment time. These results indicate that Res significantly inhibits the proliferation of 786-0 cells and exerts an antitumor effect by suppressing the expression of the VEGF gene.

Introduction

Renal cancer is one of the most prevalent malignant cancers of the urinary system in adults, and accounts for 80-85% of malignant kidney tumors. To date, there is no effective treatment modality. Numerous studies have indicated that the growth, development and metastasis of renal cancer is primarily determined by the formation of the blood vessels (1). The vascular endothelial growth factor (VEGF) plays a significant role in inducing and regulating the proliferation of vascular endothelial cells as well as in forming new vessels (2,3). Thus, the role of VEGF as an essential positive regulatory factor of angiogenesis makes it a target of research for cancer therapy. The high expression of VEGF is closely

associated with poor patient survival time, as well as with the early relapse, infiltration and lymph node metastasis of cancer. In addition to its high expression in renal cancer cells, VEGF has also been found in the serum of renal cancer patients (4,5).

Resveratrol (Res; chemical name 3,4',5-trihydroxystilbene) is a type of polyphenolic compound which mainly exists in plants, such as grapes, *eranthis hyemalis* and giant knotweed rhizomes. Res possesses multiple bioactivities, including antioxidant, anti-inflammatory, estrogen-like, growth inhibitory, immune regulatory, chemoprophylactic and antitumor activities (6). Experiments conducted *in vitro* and *in vivo* have revealed that Res can block multiple processes of carcinogenesis, and that it has inhibitory effects on the initiation, enhancement and development of tumors (7). However, numerous studies have shown that the effects of Res on cancer cells differ between cell types, and that its inhibitory effects on cell growth are only observed in certain cancer cells (8,9). To our knowledge, studies of Res have so far been primarily focused on its effects on bronchogenic, prostate, pancreatic and breast cancers, hepatoma and leukemia, while its effects on the prevention and cure of renal cancer have rarely been reported (10). Therefore, we investigated the influence of Res on the expression of VEGF and its inhibitory effect on the growth of renal cancer cells, as well as the mechanisms of action involved.

Materials and methods

Cell line and culture. The human renal cancer cell line, 786-0, was purchased from the Institute of Cell Biology, Chinese Academy of Science (CAS). The cells were maintained in RPMI-640 (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Nampa, ID, USA) and incubated in a 5% CO₂ atmosphere at 37°C. Experiments were performed at the cell logarithmic growth phase.

Chemicals. Res (purity 99%, Sigma) was dissolved in DMSO at a concentration of 100 mmol/l, and stored at -20°C in the dark. Total RNA isolation, cDNA synthesis and PCR kits were purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

MTT analysis. The assay for cell viability was performed using MTT analysis. The 786-0 cells were seeded in a

Correspondence to: Professor Huizhen Zhang, College of Public Health, Zhengzhou University, Kexue Road, Zhengzhou, Henan 450001, P.R. China
E-mail: huizhenzhangcn@126.com

Key words: resveratrol, vascular endothelial growth factor, renal cancer

Table I. The inhibitory effect of Res on the growth of 786-0 cells.

| Group (Res: $\mu\text{mol/l}$) | 24 h | | 48 h | | 72 h | |
|------------------------------------|-----------------------------------|--------|----------------------------------|--------|----------------------------------|--------|
| | OD ₅₇₀ | IR (%) | OD ₅₇₀ | IR (%) | OD ₅₇₀ | IR (%) |
| 0 | 1.2011 \pm 0.3112 | - | 1.4407 \pm 0.2312 | - | 1.6389 \pm 0.2234 | - |
| 10 | 1.0232 \pm 0.1503 ^a | 3.20 | 0.7890 \pm 0.2312 ^a | 6.71 | 0.6784 \pm 0.4321 ^a | 13.52 |
| 20 | 0.9756 \pm 1.2724 ^a | 23.32 | 0.6745 \pm 1.5100 ^b | 43.21 | 0.5462 \pm 0.3424 ^a | 54.62 |
| 40 | 0.7567.72 \pm 1.34 ^a | 46.72 | 0.5432 \pm 1.4800 ^b | 65.08 | 0.4321 \pm 1.2613 ^b | 87.71 |

OD, optical density value; IR, insulation resistance value. Compared to the control group, ^ap<0.01, ^bp<0.05.

96-well plate and incubated for 24 h. Serum-free medium was added for a 24-h incubation. The density of 786-0 cells at the logarithmic growth phase was adjusted to $1.5 \times 10^4/\text{ml}$. Cells ($200 \mu\text{l}$) were seeded in a 96-well plate and incubated for 24 h. The cells were treated with various concentrations of Res (0, 10, 20 and $40 \mu\text{mol/l}$), with 4 parallel wells to each group. The cells in 96-well plates were cultured for 24, 48 and 72 h to remove culture medium. MTT ($20 \mu\text{l}$; 5 g/l) was added to each sample and incubation continued for 4 h. After removal of the medium, DMSO was added to each well and the plates were agitated until the violet crystals were completely dissolved. After removal of the unconverted MTT, the formazan product was dissolved in isopropanol and the absorbance of formazan dye was measured at 570 nm. Viability was calculated as the percentage of absorbance relative to the control cells.

RT-PCR analysis. Total RNA was isolated from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed using the PrimeScript™ RT system kit for RT-PCR (Takara, Japan) according to the manufacturer's instructions. The primers P1 (5'-CAAGTGGTCCCAGGCTGCAC-3') and P2 (5'-CGCGAGTGTGTGTTTTCAGG-3') were designed by Primer 5.0 software according to the sequences of VEGF genes (GenBank no. AF022375.1). GAPDH was used as the internal control and the upstream and downstream primers were 5'-AAAGTGGATATTGTTGCCATC-3' and 5'-CAAATGAGCCCCAGCCTTCTCC-3', respectively. The amplified GAPDH fragment was 198 bp in length. Reaction mixture ($5 \mu\text{l}$) for each PCR contained $1 \mu\text{l}$ cDNA, $0.4 \mu\text{l}$ dNTP (2.5 mmol/l), 0.1 mM of each primer, $5 \mu\text{l}$ of 10X PCR buffer and 2.0 U Taq enzyme. The PCR reaction conditions were as follows: Pre-denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, 55°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 5 min. Amplified DNA was fractionated by electrophoresis on 1% agarose gels, and then stained with ethidium bromide and analyzed by GeneSnap software. mRNA was represented by the total absorbance obtained through the mean absorbance. The expression of VEGF mRNA was expressed as the ratio of the absorbances of VEGF and GAPDH.

Western blot analysis. Total protein samples in the 786-0 cells were extracted and determined by the BCA method.

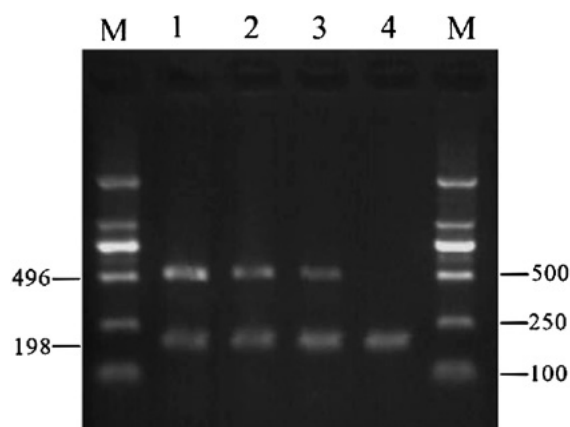


Figure 1. Regulation of the expression of VEGF mRNA by Res in the human renal cancer cell line, 786-0. Lane 1, control; lanes 2-4, cells were treated with the various concentrations of Res (10, 20 and $40 \mu\text{mol/l}$, respectively). GAPDH was used as the internal control.

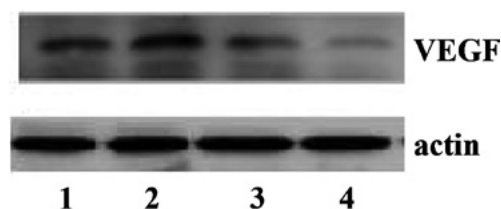


Figure 2. Regulation of the expression of the VEGF protein by Res in the human renal cancer cell line, 786-0. Lane 1, control; lanes 2-4, cells were treated with the various concentrations of Res (10, 20 and $40 \mu\text{mol/l}$, respectively). β -actin was used as the internal control.

The proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the protein bands were transferred to nitrocellulose membranes. Membranes were blocked in 5% skimmed milk for 2 h and incubated with primary antibody, rabbit anti-human VEGF antibody (diluted 1:1000) overnight at 4°C . After TBST washing 3 times (10 min for each), the membranes were incubated with peroxidase-conjugated goat anti-rabbit IgG (diluted 1:2500) for 1 h at room temperature. After TBST washing 3 times (10 min for each), the membranes were stained using ECL and then exposed to autoradiography. The films were scanned and analyzed by the gel image analysis system.

Statistical analysis. Data were represented as $\bar{x} \pm s$ and statistically analyzed using SPSS 12.0 software. The results were analyzed with one-way ANOVA analysis.

Results

Inhibitory effect of Res on the growth of 786-0 cells. The inhibitory effect of Res on the growth of 786-0 cells was detected by the MTT method. The results revealed that Res had an inhibitory effect on 786-0 cells. However, this effect varied according to the concentrations used. The effect was directly proportional to the concentration as well as to the action time, and was thus time- and dose-dependent (Table I).

Effect of Res on the expression of VEGF. The purity of total extracted RNA was determined by an ultraviolet spectrophotometer. A_{260}/A_{280} values ranged between 1.9 and 2.0, satisfying the experimental requirement.

PCR product gel electrophoresis was carried out to detect the expression of VEGF in 786-0 cells, and the results revealed that compared to the control group, the expression of VEGF was markedly decreased in the 10 and 20 $\mu\text{mol/l}$ Res groups, while no expression of VEGF was detected in the 40 $\mu\text{mol/l}$ group (Fig. 1).

Results obtained by the gel image analysis system were processed by statistical software. Within a certain range of concentrations, Res exhibited inhibitory effects on the expression of VEGF in the Eca109 cell line.

Western blot analysis was used to detect the effects of various concentrations of Res (10, 20 and 40 $\mu\text{mol/l}$), on VEGF protein expression. After 24 h, VEGF protein expression in the groups with various concentrations of Res was markedly decreased ($P < 0.01$). VEGF protein expression exhibited a decreasing tendency as the Res concentration increased (Fig. 2).

Discussion

Res has been widely studied due its inhibitory effect on tumors. Zhou *et al* adopted flow cytometry to monitor its revulsive effect on the apoptosis of renal cancer (Eca109) cells and measured the expression of survivin, bax and bcl-2 genes related to Eca109 cell apoptosis (10). In our study, MTT analysis was

used. The results revealed that Res had an inhibitory effect on the proliferation of renal cancer cells, and that this effect was directly proportional to the concentration and action time in a time- and dose-dependent manner. The RT-PCR experiment confirmed that Res had an inhibitory effect on the expression of VEGF in renal cancer 786-0 cells at certain concentrations. The above results indicate that Res has an antitumor effect, the mechanism of which is possibly related to its inhibitory effect on the expression of VEGF. Our results could provide new data to facilitate the understanding of the antitumor mechanism of Res, and could lead to the development of new clinical treatment modalities for renal cancer.

References

1. Yu DC, Chen J, Sun XT, *et al*: Mechanism of endothelial progenitor cell recruitment into neo-vessels in adjacent non-tumor tissues in hepatocellular carcinoma. *BMC Cancer* 10: 435, 2010.
2. Dvorak HF, Brown LF, Detmar M, *et al*: Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 146: 1029-1039, 1995.
3. Ranieri G, Patruno R, Ruggieri E, *et al*: Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. *Curr Med Chem* 13: 1845-1857, 2006.
4. Ljungberg BJ, Jacobsen J, Rudolfsson SH, *et al*: Different vascular endothelial growth factor (VEGF), VEGF-receptor 1 and -2 mRNA expression profiles between clear cell and papillary renal cell carcinoma. *BJU Int* 98: 661-667, 2006.
5. Jacobsen J, Grankvist K, Rasmuson T, *et al*: Expression of vascular endothelial growth factor protein in human renal cell carcinoma. *BJU Int* 93: 297-302, 2004.
6. Aggarwal BB, Bhardwaj A, Aggarwal RS, *et al*: Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res* 24: 2783-2840, 2004.
7. Kundu JK, Chun KS, Kim SO, *et al*: Resveratrol inhibits phorbol ester-induced cyclooxygenase-2 expression in mouse skin: MAPKs and AP-1 as potential molecular targets. *Biofactors* 21: 33-39, 2004.
8. Aziz MH, Kumar R and Ahmad N: Cancer chemoprevention by resveratrol: *In vitro* and *in vivo* studies and the underlying mechanisms. *Int J Oncol* 23: 17-28, 2003.
9. Jiang H, Zhang U, Jarret K, *et al*: Resveratrol-induced apoptotic death in human U251 glioma cells. *Mol Cancer Ther* 4: 554-561, 2005.
10. Zhou HB, Yan Y, Sun YN, *et al*: Resveratrol induces apoptosis in human esophageal carcinoma cells. *World J Gastroenterol* 9: 408-411, 2003.