Resveratrol promotes regression of renal carcinoma cells via a renin-angiotensin system suppression-dependent mechanism

JIANCHANG LI*, MINGNING QIU*, LIEQIAN CHEN, LEI LIU, GUOBIN TAN and JIANJUN LIU

Laboratory of Urology, Guangdong Medical University, Zhanjiang, Guangdong 524001, P.R. China

Received July 2, 2015; Accepted October 11, 2016

DOI: 10.3892/ol.2016.5519

Abstract. The aim of the present study was to investigate the effect of resveratrol on renal carcinoma cells and explore possible renin-angiotensin system-associated mechanisms. Subsequent to resveratrol treatment, the cell viability, apoptosis rate, cytotoxicity levels, caspase 3/7 activity and the levels of angiotensin II (AngII), AngII type 1 receptor (AT1R), vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) were evaluated in renal carcinoma cells. The effects of AngII, AT1R, VEGF and COX-2 on resveratrol-induced cell growth inhibition and apoptosis were also examined. The results indicated that resveratrol treatment may suppress growth, induce apoptosis, and decrease AngII, AT1R, VEGF and COX-2 levels in renal carcinoma ACHN and A498 cells. In addition, resveratrol-induced cell growth suppression and apoptosis were reversed when co-culturing with AT1R or VEGF. Thus, resveratrol may suppress renal carcinoma cell proliferation and induce apoptosis via an AT1R/VEGF pathway.

Introduction

Resveratrol (molecular formula, C14H12O3; CAS number, 501-36-0) is a non-toxic phytoalexin antioxidant and an effective anticancer compound that can be extracted from grapes, red wines, berries and peanuts (1), which imparts cancer chemopreventive and therapeutic responses (2,3). It is suggested to have potent antitumor properties against numerous human cancers (4,5). The renin-angiotensin system (RAS) is classically described as an important endocrine system that regulates blood pressure and electrolyte balance. Studies have demonstrated that RAS may be involved in numerous pathophysiological processes, such as maintaining blood pressure/blood volume homeostasis and ion-fluid balance, phylogenetic advancement, growth promotion and angiogenesis, ontogeny and phylogeny (6). RAS is also involved in several cancers, including glioblastoma multiform (7), and bladder (8) and renal (9) cancers. Previous studies have suggested that resveratrol may play anticancer roles via a RAS-dependent pathway, such as in renal (10) and bladder (8) cancer cells. Angiotensin II (AngII), which is known to be the main effector of the RAS pathway, has an important role in regulating cancer inflammation and tumor angiogenesis (11). The majority of AngII effects are mediated by 2 specific receptors subtypes, consisting of AngII type 1 receptor (AT1R) and AngII type 2 receptor (AT2R) (12), which are detected in astrocytomas (13), esophageal squamous cell carcinoma (14) and renal clear cell carcinoma (15). AT1R-mediated AngII activity plays a central role in mediating the majority of the actions of the RAS pathway (16). Vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) are important molecules in tumor-associated angiogenesis, as they activate endothelial cell metastasis and increase vascular permeability (17-19). Inhibition of VEGF suppresses tumor angiogenesis and tumor growth in vivo (20). As an important downstream regulator in RAS, VEGF is reported to be induced by AngII, by binding AT1R in pancreatic cancer cells (21). Cyclooxygenase-2 (COX-2) is a key enzyme involved in carcinogenesis and tumor progression, particularly in neoangiogenesis and lymphovascular invasion (22-24), and has also been detected as upregulated through mechanisms distinct from the VEGF axis (25-27). In the present study, the suppression of proliferation and induction of apoptosis by resveratrol was investigated in renal carcinoma cell lines. The levels of the 4 important factors in RAS, consisting of AngII, AT1R, VEGF and COX-2 were examined, and the potential mechanisms were analyzed.

Materials and methods

Cell culture and resveratrol treatment. Human renal carcinoma ACHN and A498 cell lines were purchased from Guangzhou Jennio Biological Technology Co., Ltd (Guangzhou, China). ACHN cells were cultured in high glucose Dulbecco’s modified Eagle medium (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10%
A498 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% (v/v) FBS. The two cell lines were cultured at 37°C in a 5% humidified CO₂ atmosphere. Resveratrol was purchased from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany). Resveratrol was dissolved in DMSO to create a stock solution at a concentration of 100 mM, which was subsequently diluted in culture medium to the desired concentration for experiments. DMSO was used as the vehicle control.

Enzyme-linked immunosorbent assay (ELISA). Subsequent to ACHN and A498 cells being treated with resveratrol, the medium was collected by centrifugation at 400 x g for 15 min at 4°C, and the liquid supernatant was stored at -80°C until ELISA. ACHN and A498 cells were lysed with sodium dodecyl sulfate (SDS) lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China), and total proteins were extracted at 4°C. The concentrations of AngII, AT1R, VEGF and COX-2 in the culture medium and cells were determined using a human AngII ELISA kit (catalogue no., ml003766; Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China), an AT1R ELISA kit (catalogue no., ml006788; Shanghai Enzyme-linked Biotechnology Co., Ltd.), a VEGF ELISA kit (catalogue no., ml009877; Shanghai Enzyme-linked Biotechnology Co., Ltd.) and a COX-2 ELISA Kit (catalogue no., ml006532; Shanghai Enzyme-linked Biotechnology Co., Ltd.) in accordance with the manufacturer's protocols.

Cell proliferation assay. Effects of resveratrol treatment on the cell proliferation of renal carcinoma cells were detected by Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Mashikimachi, Kumamoto, Japan) assay. ACHN and A498 cells were seeded in 96-well plates (Corning Incorporated, Corning, NY, USA) at a density of 5x10³ cells per well with 100 µl culture medium. Subsequent to allowing 24 h for adhering, resveratrol was added at varying concentrations for 12, 24 and 48 h. The culture medium was then removed and replaced with 100 µl medium containing CCK-8 reagent (10 µl; Dojindo Molecular Technologies, Inc.) in each well. The plates were incubated at 37°C for 2 h. Absorbance at 450 nm was recorded using a spectrophotometer (EnSpire 2300 Multilabel Reader; PerkinElmer, Waltham, MA, USA).

Apoptosis detection. Apoptosis cells were quantified using a fluorescein isothiocyanate Annexin V Apoptosis Detection Kit (BD Pharmingen, San Diego, CA, USA) according to the manufacturer's protocol. Cells were cultured in 6-well plates at a density of 1x10⁵ cells per well. Following 24 h growth, cells were treated with various resveratrol concentrations and harvested for the apoptosis assay. Untreated cells were used as a negative control.

Colony formation assay. Cells (1x10³ cells per well) were seeded into 6-well plates subsequent to 6 h-treatment with various concentrations of resveratrol and were cultured for 2 weeks. The number of colonies formed was counted subsequent to cells being fixed with 4% paraformaldehyde, and stained with a crystal violet staining solution (Beyotime Institute of Biotechnology).

Caspase-Glo 3/7 assays. Cells (5x10³ cells per well) were seeded onto 96-well plates and exposed to different concentrations of resveratrol. Equal volume of Caspase-Glo 3/7 reagent was subsequently added into each well and incubated for 30 min at room temperature in the dark. The luminescence was determined using a luminometer (Berthold Sirius L; Titerrek-Berthold, Pforzheim, Germany).

Cytotoxicity assay. The cytotoxicity of resveratrol was assessed using a lactate dehydrogenase (LDH) Cytotoxicity Assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Cells were cultured in 96-well plates (Corning Incorporated) and then treated with resveratrol for 24 h. The medium was collected by centrifugation at 400 x g for 5 min. Supernatant (120 µl/well) was transferred into another 96-well plate and 60 µl LDH detection reagent was added to each well, and then incubated for 30 min at room temperature in the dark. Absorbance was recorded at 490 nm with a spectrophotometer (EnSpire 2300 Multilabel Reader; PerkinElmer).

Western blot analysis. The expression of the apoptosis-associated proteins caspase 9, B-cell lymphoma 2 (Bcl-2) and Bcl-2-like protein 4 (Bax) were detected in renal carcinoma cells. Cells were lysed with radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology) and then total proteins were extracted at 4°C. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA). Membranes were then blocked using 5% non-fat milk in Tris-buffered saline and Tween 20 (TBS-T) at room temperature for 1 h, and the membranes were then probed with rabbit anti-human caspase 9 (catalog no., 9502; dilution, 1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA), Bax (catalog no., 2772; dilution, 1:1,000; Cell Signaling Technology, Inc.) and Bcl-2 rabbit monoclonal antibodies (catalog no., 2876; dilution, 1:1,000; Cell Signaling Technology, Inc.). The membranes were probed with a rabbit anti-human GAPDH polyclonal antibody (catalog no., ab37168; dilution, 1:100,000; Abcam, Cambridge, UK) as a loading control. The membranes were washed 3 times with TBS-T for 5 min each time and incubated for 1.5 h with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (catalog no., E030120; dilution, 1:10,000; EarthOx Life Sciences, Millbrae, CA, USA).

Statistical analysis. ELISA, colony formation and Caspase-Glo 3/7 assays were repeated 3 times; CCK-8 and cytotoxicity assays were performed 4 times. One-way analysis of variance (SPSS 18.0; SPSS, Inc., Chicago, IL, USA) and Student's t-test (Microsoft Excel; Redmond, WA, USA) were used to evaluate the differences between 2 groups of data in all the experiments. All data were presented as the mean ± standard deviation (SD). P<0.05 was considered to indicate a statistically significant difference and P<0.01 was considered to indicate an extremely significant difference.

Results

Resveratrol suppressed growth of renal carcinoma cells. To determine the effect of resveratrol on cell proliferation in
renal carcinoma cells, the present study firstly determined cell viability by CCK-8 assay. As shown in Fig. 1A, renal carcinoma cells treated with 25, 50, 100, 150 and 200 resveratrol for 12, 24 and 48 h, cell viability was significantly inhibited in a dose- and time-dependent manner compared with cells treated with 0 µM resveratrol [ACHN cells, P<0.001 for all concentrations at 12, 24 and 48 h; A498 cells, P<0.001 for all concentrations at 12, 24 and 48 h, with the exception of 25 µM for 12 h (P=0.0017)]. For morphology analysis, untreated renal carcinoma cells grew well, whereas the cells treated with resveratrol were distorted in shape and became round and underwent apoptosis (Fig. 1B). Furthermore, following 6 h of treatment with resveratrol, colony formation assay was performed and revealed a marked decrease in colony formation compared to the control group (ACHN cells, P=0.0158 by 50 µM and P=0.0026 by 100 µM; A498 cells, P=0.0002 by 50 µM and P=0.0002 by 100 µM; Fig. 1C).

Resveratrol induced apoptosis in renal carcinoma cells. To illustrate the functions of resveratrol on apoptosis in renal carcinoma cells, cytotoxicity, caspase 3/7 activity, cell apoptosis and expression of caspase 9, Bcl-2 and Bax were determined. It was found that resveratrol significantly increased cytotoxicity (ACHN cells: 25 µM, P<0.001; 50 µM, P=0.0036; 100 µM, P=0.0014; 150 µM, P=0.0034; and 200 µM, P=0.0007; A498 cells: 25 µM, P<0.001; 50 µM, P=0.0041; 100 µM, P=0.0027; 150 µM, P=0.0032; and 200 µM P=0.0005; Fig. 2A) and caspase 3/7 activity (ACHN cells, P<0.001 for 50, 100 and 200 µM; A498 cells, P<0.001 for 50, 100 and 200 µM; Fig. 2B) in renal cancer cells compared with cells treated with 0 µM resveratrol. Results of flow cytometry assay revealed that subsequent to 24 h of treatment with resveratrol, apoptosis was induced in renal carcinoma cells (Fig. 2C). In addition, resveratrol was shown to upregulate cleaved-caspase 9 and Bax levels and downregulate Bcl-2 level in the two cell lines (Fig. 2D).
Resveratrol suppressed AngII, AT1R, VEGF and COX-2 in a dose-dependent manner. To confirm whether AngII, AT1R, VEGF and COX-2 levels would be affected by resveratrol, ACHN and A498 cells were treated with various concentrations of resveratrol for 24 h, and the levels of AngII, AT1R, VEGF and COX-2 in cells and culture medium were determined. As shown in Figs. 3 and 4, levels of AngII (ACHN cells: 50 µM, P=0.0086; 100 µM, P=0.001; and 200 µM, P<0.001; A498 cells: 50 µM, P=0.0015; 100 µM, P=0.0002; and 200 µM, P=0.0003; culture medium for ACHN cells: 50 µM, P=0.0113; 100 µM, P=0.0031; and 200 µM, P=0.00062; culture medium for A498 cells: 50 µM, P=0.0246; 100 µM, P=0.0015 and 200 µM, P=0.0007; Figs. 3A and 4A), AT1R (ACHN cells: 50 µM, P=0.0317; 100 µM, P=0.0033; and 200 µM, P=0.0013; A498 cells: 50 µM, P=0.0221; 100 µM, P=0.0031; and 200 µM, P=0.0023; culture medium for ACHN cells: 50 µM, 0.0110; 100 µM, 0.0011; and 200 µM, 0.0008; culture medium for A498 cells: 50 µM, P=0.0340; 100 µM, P=0.0019; and 200 µM, P=0.0016; Figs. 3B and 4B), VEGF (ACHN cells: 50 µM, P=0.0062; 100 µM, P=0.0068; and 200 µM, P=0.0024; A498 cells: 50 µM, P=0.0221; 100 µM, P=0.0259; and 200 µM, 0.0045); culture medium for ACHN cells: 50 µM, P=0.0341; 100 µM, P=0.0012; and 200 µM, P=0.003; culture medium for A498 cells: 100 µM, 0.0090; and 200 µM, 0.0056; Figs. 3C and 4C) and COX-2 (ACHN cells: 100 µM, 0.0246; and 200 µM, 0.0138; A498 cells: 100 µM, 0.0398; and 200 µM, 0.0259; culture medium for ACHN cells: 50 µM, 0.0406; 100 µM, 0.0097; and 200 µM, 0.0056; culture medium for A498 cells: 100 µM, 0.0065; and 200 µM, 0.0061; Figs. 3D and 4D) were significantly decreased in the two cell lines and culture mediums in a dose-dependent manner compared with cells treated with 0 µM resveratrol.
AT1R and VEGF may reverse resveratrol-induced renal carcinoma cell growth suppression and apoptosis. To determine whether AngII, AT1R, VEGF and COX-2 may reverse resveratrol-induced renal carcinoma cell regression and apoptosis, cells were incubated with AngII (2 pg/ml), AT1R (5 pg/ml), VEGF (2 pg/ml) or COX-2 (0.5 ng/ml) when exposed to resveratrol (100 µM). AT1R and VEGF were shown to reverse cell growth suppression by resveratrol (AT1R-treatment: RES vs. RES+AT1R, P=0.0442 in ACHN cells and P=0.0235 in A498A cells; VEGF-treatment: RES vs. RES+VEGF, P=0.0429 in ACHN cells and P=0.0257 in A498A cells; Fig. 5A), and cell apoptosis assay indicated a similar result with the cell growth results (AT1R-treatment: RES vs. RES+AT1R, P=0.0312 in ACHN cells and P=0.01245 in A498A cells; VEGF-treatment: RES vs. RES+VEGF, P=0.0351 in ACHN cells and P=0.0153 in A498A cells; Fig. 5B). By contrast, AngII and COX-2 played undetected roles of resveratrol-induced cell growth suppression and apoptosis.

**Discussion**

As a type of free radical scavenger and antioxidant, resveratrol is recognized as an effective anticancer compound in several types of cancer, such as leukemia (28), breast (29-32), prostate (33) and ovarian cancers (34), melanoma (35) and primary brain tumors (36). In the present study, it was found that resveratrol may suppress renal carcinoma cell growth in a time- and dose-dependent manner. The present study also
demonstrated that resveratrol induced renal carcinoma cell apoptosis by regulating apoptosis-associated proteins caspase 3/7/9, Bcl-2 and Bax. Reduced AngII, AT1R, VEGF and COX-2 levels were detected subsequent to resveratrol treatment and highlighted the importance of AT1R and VEGF in resveratrol-induced cell apoptosis.

Previously, the renin-angiotensin system (RAS) has been considered to be an endocrine system, and the function of which is limited to regulate blood pressure and electrolyte balance. However, recent studies have detected RAS in several tissues and indicated that local RAS may be involved in regulating a variety of physiological and pathological processes (7,9,37). In
the RAS. AngII is recognized as a key biological peptide (38), with 2 major specific receptors subtypes, consisting of AT1R and AT2R (39). AngII acting on AT1R has a central role in mediating the majority of the actions of the RAS (16). Studies indicated that RAS was involved in biological activities through the AngII/AT1R pathway (39-41). In the present study, the levels of AngII and AT1R were determined subsequent to resveratrol treatment. The results demonstrated that AngII and AT1R were downregulated in the cells and the culture medium. As VEGF and COX-2 were important downstream regulators in RAS (42-45), it was found that VEGF and COX-2 were inhibited in resveratrol-treated cells and this result is similar to a previous study (29). The present data suggested that RAS suppression would be an important event during resveratrol-induced renal carcinoma cell apoptosis.

RAS was demonstrated to be involved in tumor occurrence and development (46-48). For example, AngII was able to promote epithelial-to-mesenchymal transition in intrahepatic cholangiocarcinoma (38), and angiotensin-converting enzyme inhibitor suppresses growth of colorectal cancer cells (49). AngII was demonstrated to induce VEGF in pancreatic cancer cells through binding AT1R and extracellular signal-regulated kinase 1/2 signaling; in addition, AngII/AT1R may increase expression of VEGF (21). AT1R was identified as upregulated in renal carcinoma and in urogenital cancer in a previous study (40). COX-2 was detected as upregulated in hypoxic conditions and induced angiogenesis through mechanisms distinct from the VEGF axis (25-27). As a type of anti-cancer drug, resveratrol was reported to act via a RAS-dependent pathway (10). In the present study, the effects of AngII, AT1R, VEGF and COX-2 on resveratrol-induced cell growth suppression and apoptosis in renal carcinoma were investigated. It was found that AngII and VEGF reverse resveratrol-induced renal carcinoma cell growth suppression and apoptosis; however, AngII and COX-2 had no significant effect on resveratrol-induced cell growth suppression and apoptosis. The present results suggest that AT1R and VEGF are the critical factors during resveratrol-induced renal carcinoma cell proliferation suppression and apoptosis.

In summary, the present study examined the effect of resveratrol on renal carcinoma cell growth, suppression and apoptosis and investigated the potential mechanism through RAS. The results indicated that resveratrol may inhibit cell growth, induce apoptosis, and decrease AngII, AT1R, VEGF and COX-2 production in renal carcinoma cells. However, AT1R and VEGF may reverse the functions of resveratrol on renal carcinoma cells. Additional studies should be conducted to demonstrate the exact mechanism underlying the resveratrol-induced cell growth, suppression and apoptosis, which may be, at least partially, through a RAS-associated pathway in renal carcinoma cells.

Acknowledgements

This study was supported in part by the following grants: Scientific Research Fund of Guangdong Medical College (grant no. M2014019); Science and Technology Planning Project of Guangdong Province (grant no. 2012B031800221); and The National Natural Science Foundation of China (grant no. 81272833).
Resveratrol induces apoptosis in breast cancer

Yasae A, Chen S, Hock S, Rosato R, Dent P, Dai Y and Grant S: Resveratrol sensitizes acute myelogenous leukemia cells to histone deacetylase inhibitors through reactive oxygen species-mediated activation of the extrinsic apoptotic pathway. Mol Pharmacol 82: 1030-1041, 2012.

Singh N, Nigam M, Ranjan V, Zaidi D, Garg VK, Sharma S, Chaturvedi R, Shankar R, Kumar S, Sharma R, et al: Resveratrol as an adjunct therapy in cyclophosphamide-treated MCF-7 cells and breast tumor explants. Cancer Sci 102: 1059-1067, 2011.

Shi Y, Yang S, Troup S, Davis LS, Blackwell TS, Yull F, Breyder MD and Hao CM: Interactions of the renin-angiotensin system, Endocrine J 50: 1096, 2014.

He W, Zhang M, Zhao D, Davis LS, Blackwell TS, Yull F, Breyder MD and Hao CM: Increased dietary sodium induces COX2 expression by activating NFkB in renal medullary interstitial cells. Pflugers Arch 466: 357-367, 2014.

Fan L, Feng Y, Wan HY, Ni L, Qian YR, Guo Y, Xiang Y and Li QY: Hypoxia induces dysregulation of local renin-angiotensin system in colorectal cancer and metastasis. Int J Gastroenterol 33: 389.e1-e7, 2015.

Childers WK: Interactions of the renin-angiotensin system in colorectal cancer and metastasis. Int J Colorectal Dis 30: 749-752, 2015.

Resveratrol promotes renal carcinoma regression

LI et al: RESVERATROL PROMOTES RENAL CARCINOMA REGRESSION

22. Hoellen F, Waldmann A, Banz-Jansen C, Rody A, Heide M, Köster F, Ribbat-Iedl J, Thorns C, Gebhard M, Oberländer M, et al: Expression of cyclooxygenase-2 in cervical cancer is associated with lymphovascular invasion. Oncol Lett 12: 2351-2356, 2016.

23. Ali-Fehmi R, Morris RT, Bandyopadhyay S, Che M, Schimp V, Malone JM Jr and Munkarah AR: Expression of cyclooxygenase-2 in advanced stage ovarian serous carcinoma: Correlation with tumor cell proliferation, apoptosis, angiogenesis, and survival. Am J Obstet Gynecol 192: 819-825, 2005.

24. Subbaramaiah K and Dannenberg AJ: Cyclooxygenase 2: A molecular target for cancer prevention and treatment. Trends Pharmacol Sci 24: 96-102, 2003.

25. Schmedtje JF Jr, Jr YS, Liu WL, DuBois RN and Runge MS: Hypoxia induces cyclooxygenase-2 via the NF-kappaB p65 transcription factor in human vascular endothelial cells. J Biol Chem 272: 601-608, 1997.

26. Lee JJ, Natsuizaka M, Ohashi S, Wong GS, Takaoka M, Michaylira CZ, Budo T, Tobias JW, Kanai M, Shirakawa Y, et al: Hypoxia activates the cyclooxygenase-2-prostaglandin E synthase axis. Carcinogenesis 31: 427-434, 2010.

27. Rüegg C, Dormond O and Mariotti A: Endothelial cell integrins and COX-2: Mediators and therapeutic targets of tumor angiogenesis. Biochim Biophys Acta 1654: 51-67, 2004.

28. Nakagawara H, et al: Angiotensin II type 1 receptor antagonist as an angiogenic inhibitor in prostate cancer. Prostate 67: 41-49, 2007.

29. Miyajima A, Kikuchi E, Kosaka T and Oya M: Angiotensin II type 1 receptor antagonist as an angiogenic inhibitor in urogenital cancer. Rev Recent Clin Trials 4: 75-78, 2009.

30. Miyajima A, Kosaka T, Asano T, Seto K, Kawai T and Hayakawa M: Angiotensin II type I antagonist prevents pulmonary metastasis of murine renal cancer by inhibiting tumor angiogenesis. Cancer Res 62: 4176-4179, 2002.

31. de Haas S, Delmar P, Bansal AT, Moisse M, Miles DW, Leigh N, Escudier B, Van Cutsem E, Carmeliet P, Scherjer SJ, et al: Genetic variability of VEGF pathway genes in six randomized phase III trials assessing the addition of bevacizumab to standard therapy. Angiogenesis 17: 909-920, 2014.

32. Mittal K, Koon H, Elson P, Troizzi P, Dowlati A, Chen H, Borden EC and Rini BI: Dual VEGF/VEGFR inhibition in advanced solid malignancies: Clinical effects and pharmacodynamic biomarkers. Cancer Biol Ther 15: 975-981, 2014.

33. Chang WS, Liao CH, Miao CE, Wu HC, Hou LL, Hsiao CL, Ji HX, Tsai CW and Bau DT: The role of functional polymorphisms of cyclooxygenase 2 in renal cell carcinoma. Anticancer Res 34: 5481-5486, 2014.

34. He W, Zhang M, Zhao D, Davis LS, Blackwell TS, Yull F, Breyder MD and Hao CM: Increased dietary sodium induces COX2 expression by activating NFkB in renal medullary interstitial cells. Pflugers Arch 466: 357-367, 2014.

35. Fan L, Feng Y, Wan HY, Ni L, Qian YR, Guo Y, Xiang Y and Li QY: Hypoxia induces dysregulation of local renin-angiotensin system in colorectal cancer and metastasis. Int J Gastroenterol 33: 10562-10573, 2014.

36. Ino K, Shibata H, Kajiyama H, Nawa A, Nomura S and Kikkawa F: Angiotensin II enhances epithelial-to-mesenchymal transition through the interaction between activated hepatic stellate cells and the stromal cell-derived factor-1/CXCR4 axis in intrahepatic cholangiocarcinoma. Int J Oncol 41: 573-582, 2012.