Therapeutic effect of Resveratrol in the treatment of osteoarthritis via the MALAT1/miR-9/NF-κB signaling pathway

GUOPENG ZHANG1, HUA ZHANG1, WULIN YOU2, XIAOCHEN TANG3, XIUFANG LI4 and ZHENGFENG GONG1

1Department of Orthopedics and Traumatology, Nanjing University of Chinese Medicine, Nanjing, Jiangsu 210023; 2Department of Orthopedics and Traumatology, Wuxi Hospital of Chinese Medicine, Wuxi, Jiangsu 214001; 3Department of Orthopedics and Traumatology, Suzhou Hospital of Chinese Medicine, Suzhou, Jiangsu 215009; 4Department of Science and Education, Xuyi People’s Hospital, Huaian, Jiangsu 211700, P.R. China

Received April 29, 2018; Accepted December 21, 2018

DOI: 10.3892/etm.2020.8471

Abstract. The aim of the current study was to explore the role of Resveratrol (Res) in osteoarthritis (OA) and its underlying mechanism. Reverse transcription-quantitative polymerase chain reaction and western blot analysis were used to determine the relative expression levels of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), microRNA-9 (miR-9), nuclear factor kappa B subunit 1 (NF-κB1), interleukin (IL)-6, matrix metalloproteinase 13 (MMP-13) and caspase-3 in vitro and in the in vivo model of OA, as well as examining the effect of Res on MALAT1, miR-9 and NF-κB1, IL-6, MMP-13 and caspase-3 expression levels. Immunohistochemical analysis was performed to examine the expression of NF-κB1 and MMP-13 protein levels in the in vivo model of OA. Dual-luciferase reporter assays were used to confirm the regulatory relationship between miR-9 and MALAT1 and NF-κB1, as well as examining the effect of Res on the transcriptional activation of MALAT1 promoter. Furthermore, the effect of Res on cell proliferation in vitro was examined by MTT assay. The relative mRNA expression levels of MALAT1 and NF-κB1 were significantly increased, while miR-9 expression was significantly decreased in the OA group compared with the sham group. Treatment with Res partially reversed the effects of OA on MALAT1, NF-κB1, IL-6, MMP-13 and caspase-3 expression levels. Similarly, the relative protein expression levels of NF-κB1, IL-6, MMP-13 and caspase-3 were significantly increased in the OA group compared with the sham group; however, treatment with Res partially reversed the effects of OA on the protein expression levels of NF-κB1, IL-6, MMP-13 and caspase-3. MALAT1 and NF-κB1 were identified as potential target genes of miR-9, and dual-luciferase assays were used to examine the effect of miR-9 on the luciferase activity of 3’UTR MALAT1 and NF-κB1. Treatment with Res suppressed the transcriptional activation of the MALAT1 promoter, thereby inhibiting MALAT1 expression. Additionally, the relative expression level of miR-9 significantly increased following treatment with Res in a dose-dependent manner, while the relative protein expression levels of NF-κB1, IL-6, MMP-13 and caspase-3 significantly decreased following treatment with Res compared with the control. Furthermore, treatment with Res significantly increased the growth rate of chondrocytes in a dose-dependent manner compared with the control. Taken together, these results suggest that direct targeting of the MALAT1/miR-9/NF-κB1/IL-6, MMP-13/caspase-3 axis may be a novel therapeutic strategy for the treatment of OA.

Introduction

Osteoarthritis (OA) is the most common form of rheumatic disease with the highest rate of incidence, which ultimately leads to limited joint mobility, chronic pain and disability (1). At the cellular level, OA is characterized by decreased tissue cellularity and damage to the extracellular matrix (1). It was previously demonstrated that apoptosis in chondrocytes was enhanced in the articular cartilage of patients with OA. Apoptotic cells in the cartilage of patients with OA were identified by the detection of DNA strand breaks using TUNEL assay, which identified high levels of apoptotic cells in the zones of cartilage known as the superficial and middle zones (2).

Resveratrol (Res) can regulate the expression of several intracellular signaling proteins and it is known to be associated with specific anti-inflammatory properties (3,4). In addition, Res can regulate cell proliferation while preventing inflammation and apoptosis in both the chronic and acute phases of OA (5). Res reduces the morphological changes of chondrocytes and inhibits the induction of the pro-inflammatory cytokine interleukin (IL)-1β (4). Furthermore, Res suppresses nuclear factor kappa B subunit 1 (NF-κB1)-dependent pro-inflammatory signaling and inhibits membrane-bound and mature IL-1β production in chondrocytes (3). By contrast, in vitro studies demonstrated that IL-1β inhibits the chondrocyte proliferation (3-5).
Until recently, non-coding RNAs (ncRNAs) were considered to have generic intracellular roles (6). Ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) are involved in the translation of mRNA, whereas small nuclear RNAs (snRNAs) participate in RNA splicing and small nuclear RNAs (snRNAs) mediate rRNA modification (6). Previous studies demonstrated that long non-coding RNAs (lncRNAs), RNAs >200 nucleotides in length with no or limited protein-coding ability (6,7), can serve crucial roles in several types of human cancer (8-10). In addition, lncRNAs may function to regulate gene expression at both the transcriptional and post-transcriptional levels based on genetic and epigenetic mechanisms (11,12). Furthermore, associations between lncRNAs and OA were previously investigated. Xing et al (13) identified 121 lncRNAs that were up- or downregulated in OA. MicroRNAs (miRs) are small non-coding RNA molecules derived from the introns and exons of both protein-coding and non-coding transcripts transcribed by RNA polymerase II (13-15). In addition, processed pseudogenes can activate certain miRs (16).

A previous study demonstrated that treatment with Res downregulated the expression of MALAT1, and as an lncRNA, MALAT1 can function as a molecular sponge of miR-9 (17). In addition, miR-9 can directly target NF-κB, and as an inflammatory cytokine NF-κB can induce apoptosis in chondrocytes contributing to the development of OA (18). In the current study, to explore the role of Res in OA and its underlying mechanism, the in vivo model of OA was established and the effect of Res was examined in vitro and in the in vivo model of OA. PCR techniques and western blot analysis, immuno-histochemical analysis, dual-luciferase reporter assays were performed to study underlying mechanisms, while MTT assay was used to study the effect of Res on cell proliferation in vitro. Res treatment was indicated to inhibit MALAT1 and modulate MALAT1/miR-9/NF-κB signaling pathway.

Materials and methods

Animals and experimental design. A total of 30 male C57BL/6 mice (age, 10 weeks; weight, 20-30 g) were purchased from the Shanghai Laboratory Animal Centre, Chinese Academy of Sciences (Shanghai, China). The mice were divided into three groups: Sham surgery (sham group, n=10), OA with vehicle injection (OA group, n=10) and OA with Res treatment (OA + Res group, n=10). Following 7 days acclimatization, OA was induced using the destabilizing medial meniscus (DMM) model (18). Briefly, mice were anesthetized with pentobarbital (50 mg/kg IP) and an incision was made in the right knee. The joint capsule immediately medial to the patellar tendon was incised and the joint capsule was opened using microsurgical scissors. DMM was achieved by sectioning the medial meniscotibial ligament with microsurgical scissors. Mice were sacrificed and knee joints were harvested.

Cell culture and transfection. Mouse chondrocytes (ATCC® CRL-12424™) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Chondrocytes were cultured in Dulbecco's modified Eagle medium (DMEM)/F12 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS, 0.1 mg/ml streptomycin and 1,000 U/ml penicillin (Thermo Fisher Scientific, Inc.) and maintained in a 5% CO₂-humidified incubator at room temperature. Chondrocytes were grown to 70% confluence and treated with 15 or 30 µM Res prior to transfection with miR-9 mimic or scramble control which were manufactured by Shanghai GenePharma Co., Ltd. using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Each experiment was performed in triplicate.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from tissue samples and chondrocytes using the mirVana™ miRNA Isolation kit (Ambion; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China), followed by qPCR. For miR-9 and MALAT1 expression, qPCR was performed using the TaqMan microRNA assay (Applied Biosystems; Thermo Fisher Scientific, Inc.). For NF-κB1 expression, qPCR was performed using the SYBR® Premix Ex Taq™ kit (Takara Biotechnology Co., Ltd., Dalian, China), followed by qPCR. The relative miR-9, MALAT1 and NF-κB1 expression levels were quantified using the 2^(-ΔΔCq) method (19). U6 and GAPDH mRNA were used as endogenous controls for miR-9, MALAT1 and NF-κB1, respectively. The primer pairs used were as follows: miR-9 forward, 5'-GGTCTTGTGTTATCTAGCTGTATGA-3' and reverse, 5'-3CGATGCTGCTGCTTTGAGT-3'; MALAT1 forward, 5'-CAGACCCCAACAGCTTTACAG-3' and reverse, 5'-AGA CCATCCCAAAATGCTTTCA-3'; NF-κB1 forward, 5'-CAAGGAGGGGAGCT-3' and reverse, 5'-CCCCCA GAGCCTCCACC-3'; U6 forward, 5'-CTCGCTTCGGCA GCACA-3' and reverse, 5'-AAGCTTCAGAACATTTGC GT-3'; GAPDH mRNA forward, 5'-TGACTTACACGCGA CACCCA-3' and reverse, 5'-CAACCTGTTGCTGTAGCC AAA-3'. The thermocycling conditions were as follows: 94°C for 5 min, 30 cycles of 94°C for 30 sec and 60°C for 30 sec and 72°C for 30 sec, 72°C for 10 min. Each experiment was performed in triplicate.
Cell proliferation was examined in chondrocytes by MTT assay following treatment with Res. Following a 48 h incubation, 0.5 mg/ml MTT (Sigma-Aldrich; Merck KGaA) was added to chondrocytes and incubated at 37°C for 4 h. Following incubation, culture medium was removed and 150 µl DMSO was added into each well (48-well plate at a density of 1x10^3 cells/well). The absorbance was measured at a wavelength of 490 nm using a multi-mode microplate reader (CHAMELEON™ V; Hidex, Turku, Finland) to analyze cell survival. All experiments were performed in triplicate.

**Dual-luciferase reporter assay.** The mutant 3'UTR of MALAT1/NF-κB1 was generated by mutating the miR-9 binding site sequence in the wild-type 3'UTR of MALAT1/NF-κB1. The wild-type or mutant 3'UTR of MALAT1/NF-κB1 were PCR amplified and cloned into the pRL-TK reporter vector (Promega Corporation, Madison, WI, USA). Chondrocytes were seeded into 48-well plates at a density of 1x10^3 cells/ml and co-transfected with 300 ng luciferase reporter vector containing the wild-type or mutant 3'UTR of MALAT1/NF-κB1 and 20 pmol miR-9 mimic or scramble control using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following 48-h transfection, chondrocytes were lysed and cell lysates were collected. Relative luciferase activities were detected using a Dual-Luciferase Reporter Assay system (Promega Corporation). Firefly luciferase activity was normalized to Renilla luciferase activity. Each test was performed in triplicate.

**MALAT1 luciferase assay.** The promoter region of wild-type MALAT1 was PCR amplified and cloned into the pRL-TK reporter vector (Promega Corporation). Chondrocytes were seeded into 48-well plates at a density of 1x10^3 cells/ml and transfected with 300 ng luciferase reporter vector containing the promoter region of MALAT1 using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Subsequently, chondrocytes were treated with 15 or 30 µM Res for 48 h. Following 48-h treatment with Res, chondrocytes were lysed and cell lysates were collected. The relative Renilla luciferase activity was detected using a Luciferase Reporter Assay system (Promega Corporation). Each experiment was performed in triplicate.

**Western blot analysis.** Chondrocytes were washed three times with ice-cold PBS and total protein was extracted using 0.2 ml RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). Chondrocytes were incubated in lysis buffer for 30 min on ice followed by centrifugation at 18,894 x g for 20 min at 4°C. Total protein was quantified using a bicinchoninic acid assay kit (Thermo Fisher Scientific, Inc.) and 25 µg protein was separated via SDS-PAGE on a 6 or 10% gel. The separated proteins were transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA) and blocked for 1 h at 25°C with Tris-buffered saline containing 0.1% Tween™ 20 and 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) to prevent non-specific binding. The membranes were incubated with mouse primary antibodies against NF-κB1 (1:5,000; cat. no. MA5-15128), MMP-13 (1:5,000; cat. no. MA5-14247), caspase-3 (1:5,000; cat. no. MA1-91637), IL-6 (1:5,000; cat. no. M621B all Invitrogen; Thermo Fisher Scientific, Inc.) or β-actin (1:10,000; cat. no. 3700S; Cell Signaling Technology, Inc., Danvers, MA, US) for 12 h at 4°C. Following primary incubation, membranes were incubated with anti-mouse horseradish peroxidase (HRP)-labeled secondary antibodies (1:12,000; cat. no. 7076S; Cell Signaling Technology, Inc.) at room temperature for 1 h. Protein bands were visualized using RapidStep™ ECL detection reagent (EMD Millipore) and Syngene GeneGenius Gel Light Imaging system (Syngene, Frederick, MD, USA), according to the manufacturer's protocol. Each experiment was performed in triplicate.

**Immunohistochemistry (IHC).** Tissue samples were fixed with 10% formalin at 4°C for 12 h and embedded in paraffin and paraffin-embedded samples were cut into 5-mm sections, which was later put to blocking stage with 3% hydrogen peroxide for 60 min at room temperature. For antigen retrieval, tissue sections were incubated with 0.01 M sodium citrate (pH 6) in a microwave oven for 10 min. Following antigen retrieval, tissue sections were incubated with mouse primary antibodies against NF-κB1 (cat. no. PA5-17654; 1:500) and MMP-13 (cat. no. MA5-14238; 1:500; both Invitrogen; Thermo Fisher Scientific, Inc.) for 12 h at 4°C. Following primary incubation, tissue sections were incubated with HRP-labeled secondary antibodies (cat. no. 7074S; 1:1,000; CST, Danvers, MA, US) for 1 h at room temperature. Subsequently, tissue sections were stained with hematoxylin (Dako Cytomation, Glostrup, Denmark) at 37°C for 2 h. Dimethyl benzene was used to mount the tissue sections and images were captured using a light microscope (magnification, x400). Two independent pathologists scored the staining intensity of each protein. The staining intensity was scored as follows: no staining, 0; weak staining, 1; moderate staining, 2; and strong staining, 3. Each experiment was performed in triplicate.

**Statistical analysis.** Data were presented as the mean ± standard deviation. All statistical analyses were performed using SPSS software (version 16.0; SPSS, Inc., Chicago, IL, USA). The difference between two groups was analyzed using a two-tailed Student's t-test, whilst the difference among three or more groups was analyzed using one-way analysis of variance and Scheffe's test was used as a post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**MALAT1, miR-9 and NF-κB1 expression in the in vivo model of OA.** As shown in Fig. 1, the relative expression levels of MALAT1, miR-9 and NF-κB1 were analyzed in tissue samples from mice in the sham, OA and OA + Res groups. The relative mRNA expression levels of MALAT1 and NF-κB1 were significantly increased, while miR-9 expression was significantly decreased in the OA group compared with the sham group (Fig. 1B-D). Meanwhile, compared with OA group, treatment with Res partially reversed the effects of OA on the mRNA expression levels of MALAT1, miR-9 and NF-κB1.

**NF-κB1, IL-6, MMP-13 and caspase-3 expression in the in vivo model of OA.** The relative protein expression levels of NF-κB1, IL-6, MMP-13 and caspase-3 were determined by...
western blot analysis in tissue samples from mice in the sham, OA and OA + Res groups (Fig. 2). The NF-κB1, IL-6, MMP-13 and caspase-3 protein expression levels were significantly increased in the OA group compared with the sham group (Fig. 2B-E). However, treatment with Res partially reversed the effects of OA on the protein expression levels of NF-κB1, IL-6, MMP-13 and caspase-3.

**Immunohistochemical analysis of NF-κB1 and MMP-13 in the in vivo model of OA.** IHC was performed to examine the protein levels of NF-κB1 and MMP-13 in tissue samples from mice in the sham, OA and OA + Res groups. As shown in Fig. 3, strong NF-κB1 staining was observed in the OA group compared with the sham group. Similarly, strong MMP-13 staining was observed in the OA group compared with the sham group (Fig. 4). These results suggest that the in vivo DMM-induced OA model increased NF-κB1 and MMP-13 expression. However, treatment with Res partially reversed the effects of OA on NF-κB1 and MMP-13 expression.

MALAT1 directly regulates miR-9 and miR-9 directly targets NF-κB1. The 3′UTR of MALAT1 was revealed to contain a putative binding site for miR-9 (Fig. 5A) via computational analysis using the online microRNA database (www.mirdb.org). The dual-luciferase reporter gene assay was performed to confirm the interaction between MALAT1 and miR-9 in chondrocytes. Following co-transfection with miR-9 mimic, the dual-luciferase reporter gene assay revealed that miR-9 significantly decreased the luciferase activity of wild-type MALAT1 compared with mutant MALAT1 (Fig. 5B). In addition, co-transfection with scramble control had no effect on the luciferase activity of wild-type or mutant MALAT1. To further investigate the role of miR-9 in OA, potential target genes of miR-9 were examined. Bioinformatics analysis was performed using the online microRNA database (www.mirdb.org) to identify NF-κB1 as a putative target gene of miR-9 (Fig. 5C). Following co-transfection with miR-9 mimic, the dual-luciferase reporter gene assay revealed that miR-9 significantly decreased the luciferase activity of wild-type 3′UTR NF-κB1 compared with mutant NF-κB1 (Fig. 5D), while co-transfection with scramble control had no effect on the luciferase activity of wild-type or mutant NF-κB1. Taken together, these results suggest MALAT1 directly regulated miR-9, and NF-κB1 was confirmed as a target gene of miR-9.
Res influences the transcriptional activity of the MALAT1 promoter. To further explore the underlying mechanism of MALAT1 in OA, a dual-luciferase reporter construct driven by MALAT1 promoter was examined in chondrocytes following treatment with Res. The luciferase reporter gene assay revealed that the transcriptional activation of the MALAT1 promoter was significantly decreased following treatment with Res in a dose-dependent manner compared with control (Fig. 6A). In addition, the relative mRNA expression level of MALAT1 significantly decreased following treatment with Res in a dose-dependent manner compared with control (Fig. 6B). Taken together, these results suggest that treatment with Res suppressed the transcriptional activity of the MALAT1 promoter thereby inhibiting MALAT1 expression.
Resveratrol can treat osteoarthritis by reducing chondrocyte apoptosis

Resveratrol (3,4',5-trihydroxystilbene) is a potent and selective inhibitor of NF-κB activation (18-20). In addition, Res inhibits cyclooxygenase 2 (COX-2) transcription and activity in human mammary epithelial cells (21,22). As a polyphenolic phytoestrogen, Res can activate sirtuin 1, expression of which was demonstrated previously to be inhibited in OA. In addition, Res can promote the differentiation of OA and therefore Res may be beneficial in maintaining healthy cartilage (23). In the current study, the effect of Res on the transcriptional activity

**Res effects miR-9 and the NF-κB1 signaling pathway in chondrocytes.** The expression levels of miR-9, NF-κB1, IL-6, MMP-13, caspase-3 were detected in cells following treatment with different doses (15 and 30 µM) of Res. The relative expression level of miR-9 was significantly increased, whilst the mRNA expression level of NF-κB1 was significantly decreased following treatment with Res in a dose-dependent manner compared with control (Fig. 7A and B). Similarly, the protein expression levels of NF-κB1, IL-6, MMP-13 and caspase-3 were significantly decreased following treatment with Res compared with control (Fig. 7C-G).

**Res promotes cell proliferation in mouse chondrocytes.** Following treatment with various doses (15 and 30 µM) of Res, cell viability was determined by MTT assay. The growth rate of chondrocytes significantly increased following treatment with Res in a dose-dependent manner compared with control (Fig. 8). These results suggest that treatment with Res can significantly increase chondrocyte proliferation.

**Discussion**

As an abundant phytoalexin found in grape skins and red wine, resveratrol (3,4',5-trihydroxystilbene) is a potent and selective inhibitor of NF-κB activation (18-20). In addition, Res inhibits cyclooxygenase 2 (COX-2) transcription and activity in human mammary epithelial cells (21,22). As a polyphenolic phytoestrogen, Res can activate sirtuin 1, expression of which was demonstrated previously to be inhibited in OA. In addition, Res can promote the differentiation of OA and therefore Res may be beneficial in maintaining healthy cartilage (23). In the current study, the effect of Res on the transcriptional activity
Treatment with Res suppressed the transcriptional activity of the MALAT1 promoter thereby inhibited MALAT1 expression. In addition, RT-qPCR and western blot analysis were used to determine the relative expression levels of MALAT1, miR-9, NF-κB1, IL-6, MMP-13 and caspase-3 in vitro and in the in vivo model of OA, as well as examining the effect of Res. Following treatment with Res, the relative expression level of miR-9 was
significantly increased in a dose-dependent manner, whereas the protein expression levels of NF-κB1, IL-6, MMP-13 and caspase-3 were significantly decreased.

A previous study demonstrated that Res reduced the expression of MALAT1 (16). To investigate the effect of Res on MALAT1 expression in OA, a dual luciferase reporter construct driven by MALAT1 promoter was examined in chondrocytes following treatment with Res. The luciferase reporter gene assay revealed that treatment with Res significantly suppressed the transcriptional activity of the MALAT1 promoter. These results suggest that Res may directly influence the transcription of MALAT1. In the current study, the
The level of caspase-3 activity and an increased rate of apoptosis, as well as the expression of NF-kB1 and MMP-13 were significantly increased in the OA group compared with the sham group. However, treatment with Res partially reversed the effects of OA on protein expression, and MALAT1 may function as a molecular sponge of miR-9. In addition, miR-9 can negatively regulate the expression of NF-kB1, thus suggesting that a reduction in miR-9 expression may increase the expression of NF-kB and therefore inhibit cell proliferation in vivo and in vitro.

In conclusion, treatment with Res downregulates MALAT1 expression, and MALAT1 may function as a molecular sponge of miR-9. In addition, miR-9 can directly target NF-kB, and as an inflammatory cytokine NF-kB can induce apoptosis in chondrocytes contributing to the development of OA. In the current study, DMM was used to establish an in vitro model of OA, which was treated with Res and the effect of Res was examined in chondrocytes following treatment with 0, 15 or 30 µM Res for 48 h. n=3. *P<0.05 vs. control group.

Acknowledgements
Not applicable.

Funding
No funding was received.

Availability of data and materials
The data that support the findings of the present study are available from the corresponding author upon reasonable request.

Authors' contributions
GZ and ZG planned the study, HZ and WY collected the literature, GZ, HZ, WY, and ZG collected the data, XT, XL, and ZG analyzed the data, GZ and ZG prepared the manuscript and all the other co-authors approved the final manuscript.

Ethics approval and consent to participate
The current study was approved by the Institutional Ethics Committee on Animal Research at Nanjing University of Chinese Medicine.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Sasaki H, Takayama K, Matsushita T, Ishida K, Kubo S, Matsumoto T, Fujita N, Oka S, Kurosaka M and Kuroda R: Autophagy modulates osteoarthritis-related gene expression in human chondrocytes. Arthritis Rheum 64: 1920-1928, 2012.
2. Goggs R, Carter SD, Schulze-Tanzil G, Shakibaei M and Mobasher A: Apoptosis and the loss of chondrocyte survival signals contribute to articular cartilage degradation in osteoarthritis. Vet J 166: 140-158, 2003.

3. Csaki C, Keshishzadeh N, Fischer K and Shakibaei M: Regulation of inflammation signalling by resveratrol in human chondrocytes in vitro. Biochem Pharmacol 75: 677-687, 2008.

4. Csaki C, Mobasher A and Shakibaei M: Synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes: Inhibition of IL-1beta-induced NF-kappaB-mediated inflammation and apoptosis. Arthritis Res Ther 11: R165, 2009.

5. Shakibaei M, Csaki C, Nebrich S and Mobasher A: Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: Potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochem Pharmacol 76: 1426-1439, 2008.

6. Fatica A and Bozzone I: Long non-coding RNAs: New players in cell differentiation and development. Nat Rev Genet 15: 7-21, 2014.

7. St Laurent G, Wahlestedt C and Kapranov P: The Landscape of long noncoding RNA classification. Trends Genet 31: 239-251, 2015.

8. Mercer TR, Dinger ME and Mattick JS: Long non-coding RNAs: Insights into functions. Nat Rev Genet 10: 155-159, 2009.

9. Ponting CP, Oliver PL and Reik W: Evolution and functions of long noncoding RNAs. Cell 136: 629-641, 2009.

10. Ji P, Diederichs S, Wang W, Boing S, Metzger R, Schneider PM, et al: MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 22: 8031-8041, 2003.

11. Holoch D and Mouzad D: RNA-mediated epigenetic regulation of gene expression. Nat Rev Genet 16: 71-84, 2015.

12. Yoon JH, Abolsmehlen K and Gerospe M: Posttranscriptional gene regulation by long noncoding RNA. J Mol Biol 425: 3723-3730, 2013.

13. Xing D, Liang QJ, Li Y, Lu J, Jia HB, Xu LY and Ma XL: Identification of long noncoding RNA associated with osteoarthritis in humans. Orthop Surg 6: 288-294, 2014.

14. Mattick JS and Makunin IV: Small regulatory RNAs in gene regulation by long non-coding RNA. J Mol Biol 425: 3723-3730, 2013.

15. Rodriguez A, Griffiths-Jones S, Ashurst JL and Bradley A: Identification of mammalian microRNA host genes and transcript units. Genome Res 14: 1902-1910, 2004.

16. Devor EJ: Primate microRNAs miR-220 and miR-492 lie within processed pseudogenes. J Hered 97: 186-190, 2006.

17. Ji Q, Liu X, Fu X, Zhang L, Sui H, Zhou L, Sun J, Cai J, Qin J, Ren J and Li Q: Resveratrol inhibits invasion and metastasis of colorectal cancer cells via MALAT1 mediated Wnt/beta-catenin signal pathway. PLoS One 8: e78700, 2013.

18. Gu R, Liu N, Luo S, Huang W, Zha Z and Yang J: MicroRNA-9 regulates the development of knee osteoarthritis through the NF-kappaB1 pathway in chondrocytes. Medicine (Baltimore) 95: e4315, 2016.

19. Xu L, Polur I, Servais JM, Hsieh S, Lee PL, Goldring MB and Li Y: Intact pericellular matrix of articular cartilage is required for unactivated discoidin domain receptor 2 in the mouse model. Am J Pathol 179: 1338-1346, 2011.

20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

21. Manna SK, Mukhopadhyay A and 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 164: 6509-6519, 2000.

22. Estrov Z, Shishodia S, Faderl S, Harris D, Van Q, Kantarjian HM, Talpaz M and Aggarwal BB: Resveratrol blocks interleukin-1beta-induced activation of the nuclear transcription factor NF-kappaB, inhibits proliferation, causes S-phase arrest, and induces apoptosis of acute myeloid leukemia cells. Blood 102: 987-995, 2003.

23. Holmes-McNary M and Baldwin AS Jr: Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res 60: 3477-3483, 2000.

24. Subbaramaiah K, Chung WJ, Michalauert P, Telang N, Tanabe T, Inoue H, Jiang M, Pazzutto JM and Dannenberg AJ: Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. J Biol Chem 273: 21875-21882, 1998.

25. Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK and Lee SS: Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: Down-regulation of COX-2 and iNOS through suppression of NF-kappaB activation. Mutat Res 480-481: 243-268, 2001.

26. Shan T, Wang Y, Wu T, Liu C, Guo J, Zhang Y, Liu J and Xu Z: Porcine sirtuin I geneclone, expression pattern, and regulation by resveratrol. J Anim Sci 87: 895-904, 2009.

27. Salemi M, Barone C, Romano C, Scillato F, Ragalmuto A, Caniglia S, Salluzzo MG, Scutio G, Ridolfo F and Boscio P: NF-xB1 gene expression in Down syndrome patients. Neurol Sci 36: 1065-1066, 2015.

28. Song J, Kim D, Chun CH and Jin EJ: MicroRNA-9 regulates survival of chondroblasts and cartilage integrity by targeting proteogenin. Cell Commun Signal 11: 66, 2013.

29. Tomek M, Akiyama T and Dass CR: Role of Bcl-2 in tumour cell survival and implications for pharmacotherapy. J Pharm Pharmacol 64: 1695-1702, 2012.

30. Liu N, Sun Q, Chen J, Li J, Zeng Y, Zhai S, Li P, Wang B and Wang X: MicroRNA-9 suppresses uveal melanoma cell migration and invasion through the NF-xB1 pathway. Oncol Rep 28: 961-968, 2012.

31. Guo LM, Pu Y, Han Z, Liu T, Li YX, Liu M, Li X and Tang H: MicroRNA-9 inhibits ovarian cancer cell growth through regulation of NF-kappaB1. FEBS J 276: 5537-5546, 2009.

32. Baltaci SB, Mogulkoc R and Baltaci AK: Resveratrol and exercise. Biomed Rep 5: 525-530, 2016.