ORIGINAL ARTICLE

Received: November 2017
Accepted: February 2018

Resveratrol affects β-catenin and GSK-3β gene expression in Wnt-signalingpathway in HCT-116 human colorectal cancer cells

Monireh Sadat Seyyedsalehi[1], Ali Akbar Saboor-Yaraghi[1,4], Fariba Koohdani[1], Fatemehsadat Amiri[2], Marjan Norouzzadeh[1], Yas Kalikias[1], Said Talebi[5], Parvin Pasalar[3], Azar Berahmeh[4], Farnoosh Shemirani[1], Maryam Mahmoodi[1,*]

1. Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetic, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran.
3. Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences,Tehran, Iran.
4. Department of Immunology ,School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
5. Department of medical genetics Tehran University of Medical Sciences, Tehran, Iran.

***Corresponding Author:**
Maryam Mahmoodi
Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetic, Tehran University of Medical Sciences, Tehran 6447-14155, Iran.
Tel: (+98)2188955805 (internal 109)
Fax: (+98)2188984861
Email address:
mahmoodi_maryam@yahoo.com

BCCR
2018; 10(1): 22-33
www.bccrjournal.com

ABSTRACT

Background: Colorectal cancer is one of the mostly diagnosed malignancies worldwide. The main risk factors for colorectal cancer include the mutation of tumor suppressor genes or proto-oncogenes and unhealthy lifestyle. Vegetable and fruit consumption with multiple anticancer agents can reduce the risk of colon cancer. Resveratrol is a natural polyphenolic product that inhibits proliferation and induces apoptosis through several pathways. In this study the effects of resveratrol on β-catenin (CTNNB1) and GSK-3β expression in the Wnt-signaling pathway were examined and, morphology changes were analyzed in colon cancer cells with high levels of β-catenin such as HCT-116.

Methods: HCT-116 cells were seeded into 6-well plates, and the cells were treated with various concentrations of resveratrol (25, 50 and 100 μM) for 24, 48 and 72 hours respectively. Quantitative Real-time PCR examined β-catenin and GSK-3β expression and morphology changes were analyzed.

Results: The results showed that, in 25 and 50 μM concentrations, resveratrol reduced β-catenin and GSK-3β expression in 24 h (p-value; 0.001). Gene expressions were found to increase in 48 h and 72 h treatment with resveratrol in the concentrations of 50 and 100 μM respectively (p-value; 0.001).

Conclusion: considering our data, it can be concluded that low doses of resveratrol could reduce β-catenin expression, which can affect the Wnt-signaling pathway. High doses can increase the GSK-3β expression, playing a role in the destruction of β-catenin, inhibition of its accumulation in the cytoplasm and nuclear, apoptosis induction and cellular proliferation inhibition while low doses of resveratrol can decrease GSK-3β expression and suppress proliferation.

Abbreviations: LEF/TCF, lymphoid enhancing factor/T-cell factor; APC, adenomatous polyposis coli; GSK-3β, glycogen synthase kinase 3B; CK, casein kinase 1; RSV, resveratrol; DMSO, dimethyl sulfoxide; DMEM-F12, Dulbecco's Modified Eagle's Medium F-12; FBS, fetal bovine serum; CIN, chromosomal instability; PI3K, phosphatidylinositol-3-kinase; TGF-β, transforming growth factor-β; GFR, Growth Factor Receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; PTEN, Phosphatase and tensin homolog; COX, Cytochrome c oxidase; IGF-1R, insulin-like growth factor 1 (IGF-1) receptor; Akt, Protein kinase B (PKB);

Keywords: β-catenin, Colorectal cancer, CTNNB1, GSK-3β, HCT-116, Resveratrol, Wnt signaling

INTRODUCTION:

Colorectal cancer and signaling pathway

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in females and the third in males in the world[1]. The lowest incidence rates of colorectal cancer are found in Africa, whereas the highest ones are found in Australia, New Zealand, Europe, and North America[2]. The treatment approaches to colorectal cancer include surgical resection or radiotherapy combined with chemotherapy[3]. Amongst the modifiable risk factors are physical inactivity, overweight and central obesity, the Western diet (e.g., saturated fats, red and processed meat consumption, excessive alcohol consumption and perhaps refined carbohydrates) and smoking[4,5]. In addition, CRC may occur in form of one of the most studied three patterns, including familial, inherited and sporadic[6,7]. The Wnt-signaling pathway is a tightly regulated cascade. Wnt-factors, which constitute a large family of cell-signaling glycoproteins, play a key role during normal animal development[8,9]. The Wnt-signaling pathways are a group of signal transduction pathways made of proteins, which pass signals from outside of a cell through cell surface receptors to the inside of the cell. Three Wnt signaling pathways have been characterized: the canonical Wnt pathway, the noncanonical planar cell polarity pathway and the noncanonical Wnt/calcium pathway[10]. The binding of a Wnt-protein ligand activates all the three Wnt signaling pathways to a Frizzled family receptor, which passes the biological signal to the protein dishevelled inside the cell. Wnt signaling pathways use either nearby cell-cell communication (paracrine) or same-cell communication (autocrine)[10]. The canonical Wnt pathway (or Wnt/β-catenin pathway) is the Wnt pathway that acts along with β-catenin. Catenin beta-1 also known as β-catenin is a protein that, in humans, is encoded by the CTNNB1 gene. β-catenin is a dual function protein, regulating the coordination of cell-cell adhesion and gene transcription. It is a subunit of the cadherin protein complex and acts as an intracellular signal transducer in the Wnt signaling pathway[11]. Signaling through the Wnt- β-catenin–LEF/TCF complex plays crucial roles in tissue homeostasis[12]. In unstimulated cells, free cytoplasmic β-catenin is destabilized by a multi-protein complex containing tumor suppressor APC, the central scaffold protein Axin, GSK-3β and CK1 as the core components[9]. Upon cell stimulation by Wnt-ligands, the destabilization of free cytoplasmic β-catenin is inhibited. Stabilized β-catenin will be increased and translocate into the nucleus. In the nucleus, β-catenin binds to members of LEF/TCF family of transcription factors, leading to transcription/activation of Wnt-target genes that stimulate or inhibit the molecules in the cell cycle[13,14]. Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular carcinoma, colorectal carcinoma, lung cancer, malignant breast tumors, ovarian and endometrial cancer[11]. The role of GSK-3β in tumorigenesis and cancer progression is controversial; it may function as a promoter of growth and development with effects on some factors (NF-κB and AP-1), i.e. overexpression of a constitutively active form of GSK-3β increases cyclin D1 expression and induces cell cycle progression of some cancer cells, or it may act as a tumor suppressor for certain types of tumors through regulating the Wnt/ β-catenin signaling pathway[15].

According to the results of several epidemiological studies, vegetable and fruit consumption is associated with a reduced risk of colorectal cancer; multiple phytochemicals have been introduced as potential anticancer agents from commonly consumed fruit and vegetables[16]. RSV; 3, 4', 5-trihydroxy-trans-stilbene is a well-known natural polyphenolic product and a phytoalexin, produced by about 70 plant species, including grapes, peanuts, mulberries, berries, plums, and pines, and is widely present in red wine[17,18]. Resveratrol in-

take has been reported to have an anti-inflammatory and anti-oxidative impact and to be capable of improving the immunomodulatory pathways[19, 20]. Moreover, it plays a crucial role in inhibiting the growth of cancer cell lines derived from various origins such as the breast, colon, melanoma, uterine, lung and leukemia cells[17, 18]. As reported in the previous studies, resveratrol suppresses colon cancer cell proliferation and elevates apoptosis through several pathways, namely via suppression of IGF-1R/Akt/Wnt-signaling pathways[15]. The present study aimed to investigate the time- and dose-dependent manner effects of resveratrol on β-catenin and GSK-3β expression in the Wnt-signaling pathway in HCT-116 human colorectal cancer cells. In the cell line the level of B-catetin is high. A study conducted in 2013 found that in colorectal cancer cells with high levels of β -catenin, inhibitors of AKT (a class of chemotherapy drugs) may increase the FOXO3a. Typically, FOXO3a causes apoptosis in cells, but high concentrations of β -catenin within the cell causes the metastasis. The purpose of this study was to investigate the effect of resveratrol on the concentration of β -catenin by measuring changes in gene expression.

METHODS:

Chemicals and reagents

Resveratrol (Product Number: R5010) and Sodium bicarbonate (Product Number: S5761) were obtained from Sigma Aldrich (St.Louis, Mo). RSV was dissolved to the concentration of 50 μM in 100% dimethyl sulfoxide (DMSO). Dimethyl sulfoxide (DMSO) was purchased from Sigma Aldrich (St.Louis, Mo / Product Number: D2650), as a stock solution, and stored at -20˚C. The final DMSO concentrations used in the present study were ≤0.1%. Dulbecco's Modified Eagle's Medium F-12 (DMEM-F12) (Product Number: 32500032), fetal bovine serum (FBS) (Product Number: 10270106), Penicillin-Streptomycin (Product Number: 15140-122) and Trypsin/EDTA (Product Number: 25200-056) were purchased from the Gibco (Pittsburgh, PA).

Cell lines and culture conditions

Human colorectal cancer cell lines HCT-116 were purchased from national cell bank of Iran (Pasteur Institute, Iran). Cells were maintained at 37°C incubator with 5% CO2 in DMEM-F12 medium supplemented with antibiotics (100 U/ml penicillin, 100 g/ml streptomycin) and 10% (v/v) heat-inactivated FBS and sodium bicarbonate. All cell cultures were monitored routinely. The cells were kept sub-confluent and the media were changed every one to two days. All cells used were within 2 to 3 passages.

Treatment condition, RNA isolation and creating the cDNA

HCT-116 cells (6 x10^5) were seeded into 6-well plates and allowed to adhere for 24 hours. When the proliferation of the cells was 60-70%, the cells were treated with DMEM-F12/FBS %5 containing various concentrations of resveratrol (25, 50 and 100 μM) and DMSO (vehicle control) for 24, 48 and 72 hours and collected by trypsinization. Because of some of the limitations, we didn't MTT assay to confirm any toxicity of the resveratrol concentrations in the HCT-116 cell line and then to choose the best concentrations. So we reviewed several past studies[21-24] and chose the dose and timing of the intervention. At the end of each round of treatment with resveratrol, cell changes were recorded. Total RNA was isolated from the cells, using RNeasy Mini Kit according to the manufacturers' instructions (Qiagen, CA, USA / Product Number: 74134). RNA concentration and purity were checked by A260/A280 optical density ratio and NanoDrop 2000c Spectrophotometer (Thermo, USA). cDNA was generated from 1 μg RNA, using PrimeScriptTM RT reagent Kit (TA-

KARA /Cat. RR037A), and random primers, and was subjected to 40 cycles quantitative RT-PCR analysis.

Quantitative RT-PCR analysis

Quantitative Real-time PCR was done, using a SYBR Premix Ex TaqTM ⊓ (Tli RNaseH Plus), Bulk (TaKaRa-cat: RR820L) in StepOne system (Applied Biosystems, Foster City, CA). The primer sequences used for quantitative RT-PCR were as follows: β-catenin (CTNNB1) forward 5'- CGAAATCTTGCCCTTTGTCC-3', reverse 5'-GTTGTGAACATCCCGAGCTAG-3'; GSK-3β forward5'GGTCTATCTTAATCTGGTGCTGG-3', reverse 5'TGGATATAGGCTAAACTTCGGAAC-3' and β-actin as reference gene with 5'-AGCCTCGCCTTTGCCG-3' forward primer and 5'-ATCACGCCCTGGTGCCT-3' reverse primer served as an internal control. Primer sequence was designed as follows: 1) find the Proper sequence from related articles. 2) go to NCBI (National Center for Biotechnology Information) webpage: http://www.ncbi.nlm.nih.gov/ and Select BLAST engine. 3) Go to Primer-3 Software: http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi.

Statistical analysis

All experiments were done in three technical replicates, and the results were repeated at two independent biological replicates. To determine the effects of time and dose of intervention, the data were analyzed by pairwise fixed reallocation randomization test, using Relative Expression Software Tool (Rest-RG and Rest-MCS) software. Results were considered significant with P-value less than 0.05.

RESULTS:

Expression of CTNNB1 and GSK-3β in the Wnt-signaling

Effects of RSV at varying concentrations (25, 50 and 100 μM) and time points (24-48-72 h) on the expression of CTNNB1 and GSK-3β in HCT-116 cell line were determined by Quantitative real time PCR. The results showed that resverator dose- and time-dependent manner effects on the expression of these genes (**Figure. 1, 2, 3, 4**). The effect of DMSO in HCT-116 cells was compared with the control non-treated cells, and no significant differences were observed between non-treated and DMSO treated cells. Cells treated with DMSO were used as the control.

Dose-dependent effects

The effects of doses of 25, 50 and 100 μM resveratrol on the expression of β-catenin gene.

Comparison of β-catenin expression after 24 hours of treatment with 25, 50 and100 μM concentrations of resveratrol showed that β-catenin expression in 25 and 50 μM doses of resveratrol decreased (p-value; 0.001), but increasing in 100 μM. After 48 hours, GSK-3β expression increased in all of the doses of resveratrol but in 50 it was significant. Besides, GSK-3β expression elevated significantly upon treatment with the 25, 50 and 100 μM (p-value; 0.001) concentration of resveratrol after 72 h (p-value; 0.001) (**Figure 1**).

The effects of doses of 25, 50 and 100 μM resveratrol on the expression of GSK-3β gene

The results of 24-hours treatment of GSK-3β expression with resveratrol in different concentrations, i.e. 25, 50 and 100 μM; showed that GSK-3β expression in 25 and 50 μM doses of resveratrol (p-value; 0.001) decereased, but it increased in 100 μM (p-value; 0.001). After 48 hours, GSK-3β expression increased in all of the doses of resveratrol but in 50 and 100 μM it was significant. Besides, GSK-3β expression elevated significantly upon treatment with the 25, 50 and 100 μM (p-value; 0.001) concentration of resveratrol after 72 h (p-value; 0.001) (**Figure 2**).

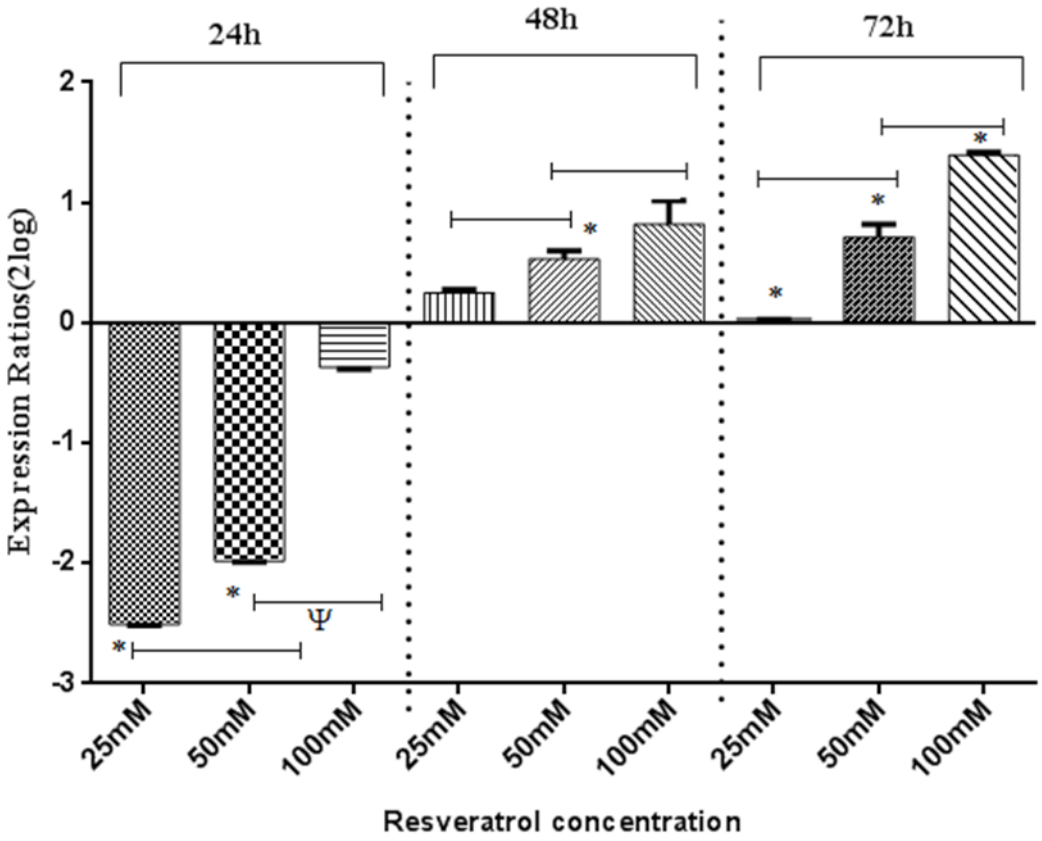

Figure 1. The effects of resveratrol (25, 50 and 100 mM) on the β-catenin (CTNNB1) gene expression in the HCT-116 cell line.
Comparison of β-catenin expression after 24 h, 48 h and 72 h of treatment with resveratrol (25, 50 and 100 μM), measured by quantitative real-time PCR and represented as amount of mRNA, normalized to actin, and graphed as 2Log of expression Ratios (y-axis). Resveratrol concentrations included 25, 50, and 100 μM (x-axis). Error bars represent SEM.

* and Ψ denote statistically significant difference (p-value < 0.05) compared to control cells (DMSO) , and previous intervention group ,respectively

Time-dependent effects:

Time-dependent (24, 48 and 72 h) manner effects of resveratrol on the expression of β-catenin gene

Besides, β-catenin expression elevated upon treatment with the 25, 50 and 100 μM concentrations of resveratrol after 72 h (**Figure 3**).

Time-dependent (24, 48 and 72 h) manner effects of resveratrol on the expression of GSK-3β gene

Results of time-dependent manner effect of resveratrol on GSK-3β expression showed that the expression of

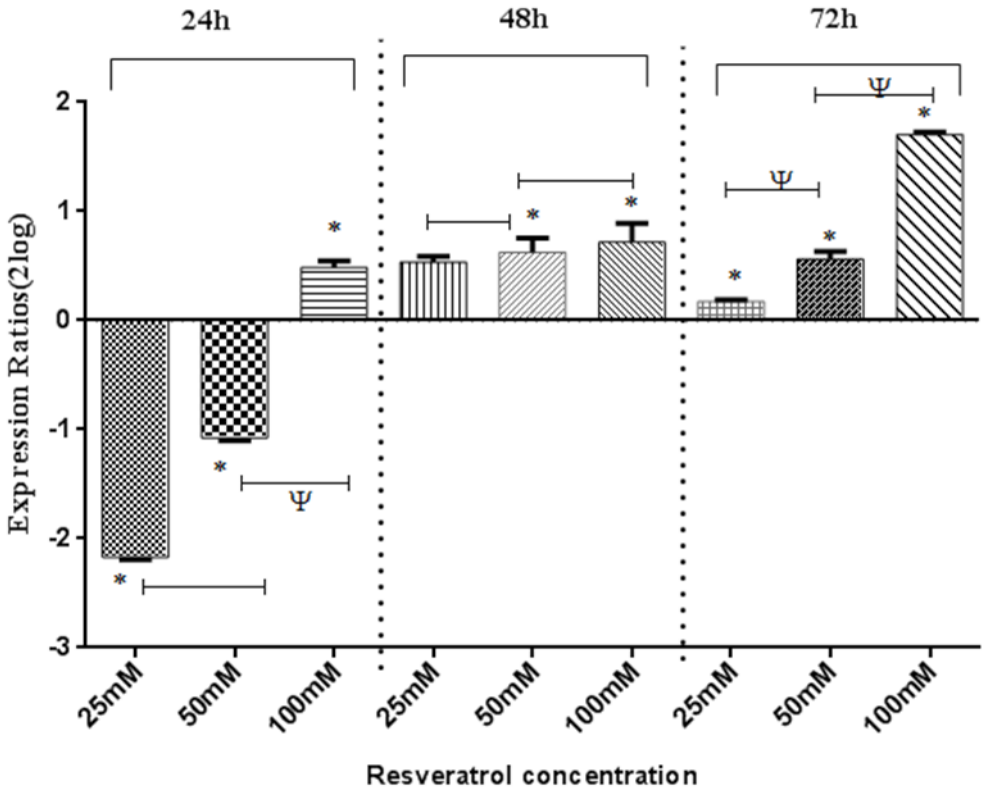

Figure 2. The effects of resveratrol (25, 50 and 100 mM) on the GSK-3β gene expression in the HCT-116 cell line.
Comparison of GSK-3β expression after 24 h, 48 h and 72 h of treatment with resveratrol (25, 50 and 100 μM), measured by quantitative real-time PCR and represented as amount of mRNA, normalized to actin, and graphed as 2Log of expression Ratios (y-axis). Resveratrol concentrations included 25, 50, and 100 μM (x-axis). Error bars represent SEM

* and Ψ denote statistically significant difference (p-value < 0.05) compared to control cells (DMSO) , and previous intervention group ,respectively

GSK-3β reduced at 25 μM and 50 μM of resveratrol in 24 h (p-value; 0.001), compared with 48 h and 72 h, but GSK-3β expression increased significantly after 72 h (p-value; 0.001) (**Figure 4**).

Morphological changes

The untreated HCT-116 cells with resveratrol were observed to be healthy with clear skeletons and spindle-like and fusiform features, whereas after resveratrol treatment, cells began to change morphologically. An increase in size, a more polygonal shape, blurring of the border between the cytoplasm and the nucleus

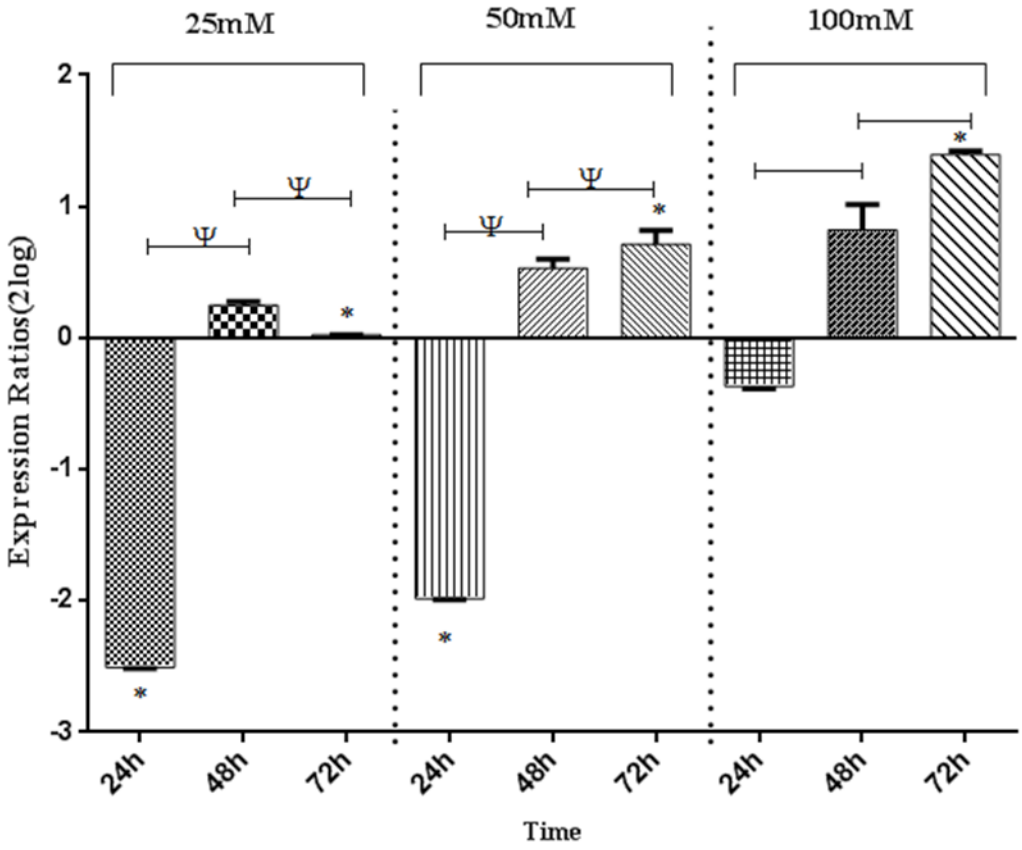

Figure 3. The effects of time 24, 48 and 72 h on the expression of β-catenin gene in the HCT-116 cell line. Comparison of β-catenin expression after treatment with 25, 50 and 100 μM between 24 h, 48 h and 72 h, measured by quantitative real-time PCR and represented as amount of mRNA, normalized to actin, and graphed as 2Log of expression Ratios (y-axis). Resveratrol concentrations included 24, 48, and 72 h (x-axis). Error bars represent SEM.

* and Ψ denote statistically significant difference (p-value < 0.05) compared to control cells (DMSO) , and previous intervention group ,respectively

and nucleus-cytoplasm fusion were the changes observed. These changes were more intuitive when the dose and duration of exposure to cells were increased by resveratrol intervention; a dose of 100 μM in 72 hours caused the cell death and removal of the cells from the floor of the plates (**Figure 5**).

DISCUSSION:

Colorectal cancer is one of the mostly diagnosed malignancies with high prevalence in western countries. The American Cancer Society reported 39,870 new cases of rectal and 101,340 cases of colon cancers and 49,380 deaths in 2011 in the United States[25]. There are

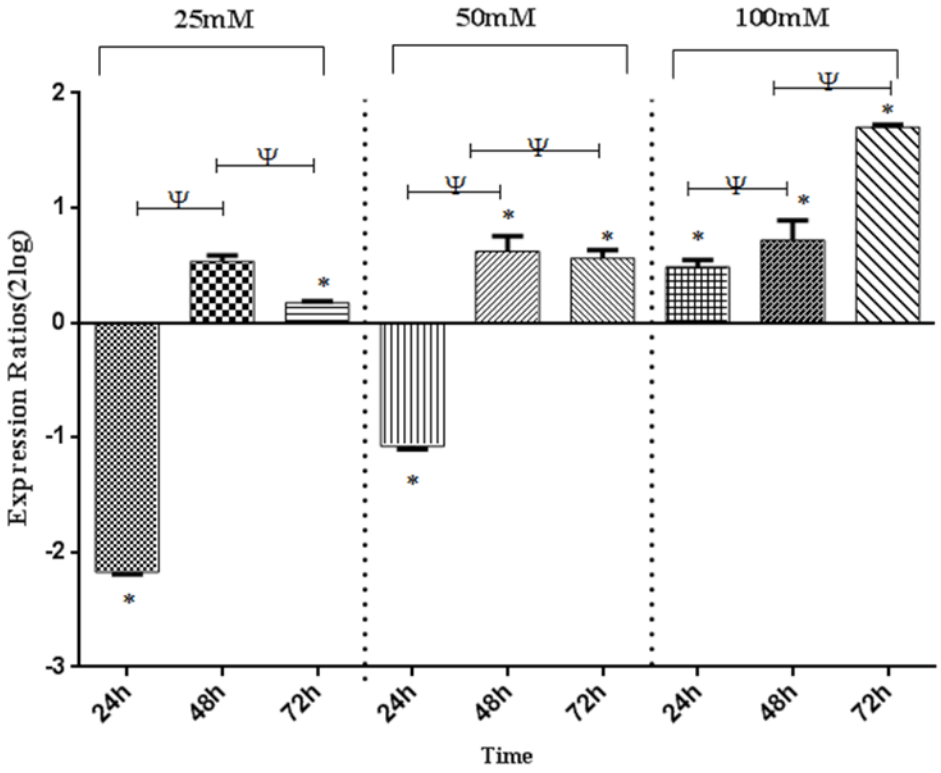

Figure 4. The effects of time 24, 48 and 72 h on the expression of GSK-3β gene in the HCT-116 cell line.

Comparison of GSK-3β expression after treatment with 25, 50 and 100 μM between 24 h, 48 h and 72 h, measured by quantitative real-time PCR and represented as amount of mRNA, normalized to actin, and graphed as 2Log of expression Ratios (y-axis). Resveratrol concentrations included 24, 48, and 72 h (x-axis). Error bars represent SEM.

* and Ψ denote statistically significant difference (p-value < 0.05) compared to control cells (DMSO) , and previous intervention group ,respectively

many pathways which are responsible for the onset and also progression of colorectal cancer, including Wnt/β-catenin signaling, TGF-β signaling, KRAS signaling, Hedgehog signaling and apoptotic signaling[26]. β-catenin performs several functions in Wnt-signaling, and most colorectal cancer cells are predisposed to the mutation of β-catenin[11]. Indeed, β-catenin is a structural protein and plays a role in cell adhesion, controlling the G1 to S phase transition in the cell cycle[12,19,20]. Also, Wnt-signaling is required for the routine renewal cycle of intestinal and colonic tissues, but its hyperactivation results from APC, GSK-3β, AXIN, and CK1α

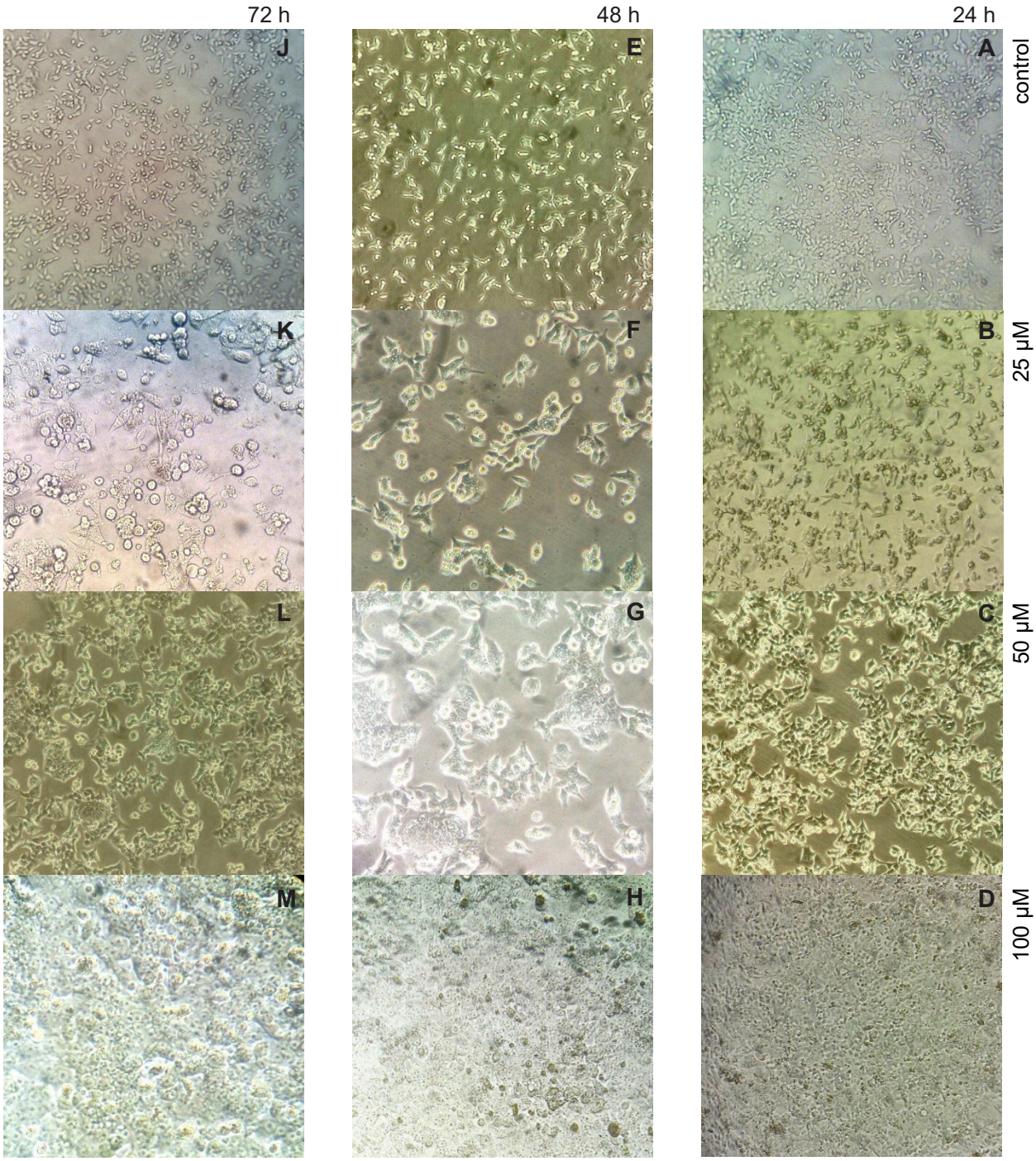

Figure 5. (A, E, J) control Group for 24 h, 48 h and 72 h, (B, F, K) the cell line treated with 25 µM of resveratrol for 24 h, 48 h and 72 h. (C, G, L) the cell line treated with 50 µM of resveratrol for 24 h, 48 h and 72 h. (D, H, M) the cell line treated with 100 µM f resveratrol for 24 h, 48 h and 72 h.

or β-catenin mutations triggers uncontrolled cell proliferation and eventually leads to cancer[27]. GSK-3β, a family of protein-serine kinases, was initially characterized as a regulator of glycogen metabolism and critical cellular functions such as mobility, gene expression and apoptosis[3, 28, 29]. Levels of GSK-3β expression and amounts of its active form in colon cancer cell lines in colorectal cancer patients are higher than those in their normal counterparts. Therefore, GSK-3β may be a ‘‘tumor promoter” for certain types of tumors. For this reason, inhibition of GSK-3β has been proposed to be an attractive therapeutic approach for the treatment of colorectal cancer[15]. This study aimed to investigate Wnt-signaling by assessing β-catenin and GSK-3β gene expression in response to resveratrol treatments in HCT-116 cells. The ameliorative effects of resveratrol on cancers have been investigated in several previous studies[6,16,22]. Resveratrol was discovered in 1940 and has been shown to suppress the proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers; breast, colorectal[30,31], pancreas, stomach, prostate, head and neck, liver, ovary, lung and cervical cancers and melanoma. Besides inhibiting proliferation, resveratrol also induces apoptosis through several pathways[32-35].

In a study, conducted in 2008, on the effects of resveratrol in a dose-dependent Wnt signaling pathway in colon cancer cells, it was found that low concentrations of resveratrol inhibits the activity of the β-catenin by reducing the accumulation of β-catenin in the nucleus and decreasing expression of regulatory factors (Imprinted genes (IGs), Pygopus)[21]. Another study in 2012 showed that resveratrol reduces target gene expression in Wnt signaling pathways, such as Cyclin D1, Axin2, ET-1, c-Myc and inhibit cell growth. In this study, the effect of resveratrol on the concentration of cytoplasmic and nuclear β-catenin wasn’t reported[22]. In 2013, Qing Ji et al. demonstrated that resveratrol through affecting MALAT1 can inhibit the accumulation of intracellular β-catenin. Resveratrol also reduced the expression of Wnt signaling products[6].Ying-Zi Liu Study in 2014 suggested that resveratrol may inhibit the signaling pathway, via effects on gene expression of β-catenin[16]. Our result showed that resveratrol in low concentration (25 μM) reduced β-catenin expression in 24 hours. At 50 μM resveratrol, gene expression in 24 h reduced, but with overtime expression it increased so that in 72 h this increase was significant. So, it could be concluded that the best dose and timing of inhibitory effect of resveratrol on the expression of β-catenin is 25 μM and 50 μM after 24 hours.

A study in 2009 on the effect of resveratrol on the signaling pathway Wnt\β-catenin in mesangial cells at varying concentrations (1, 5, 10 and 50 μM) showed that low doses of resveratrol could decrease the level of phosphorylation of GSK-3β[36]. Another study in 2010 on the effects of resveratrol on HUVECs (human umbilical vein endothelial cells) showed that at doses of 1 μM to 10 μM of resveratrol the total GSK-3β and phosphorylated GSK-3β is low. With the increase of the dose, it was observed that the total amount of GSK3B remained high and the concentration of phosphorylated GSK-3β increased in the cells[23]. Also, in another study in 2011 on the Neuro-2A (N2A) cell line at concentrations of 10, 50, 100, 200 μM of resveratrol, it was found that phosphorylates GSK-3β increased significantly at 100 μM in 2 hours[24]. We have studied the effect of resveratrol on GSK-3β gene expression. In the 24 h, 25 μM dose and 50 μM of resveratrol reduced the GSK-3β expression and 100 μM increased the expression. 50 μM and 48 h after treatment was found to increase the expression of genes.

CONCLUSION:

The results of our study uncovered a Wnt inhibitory activity of resveratrol and compared the effects of low vs high doses of resveratrol treatment in HCT-116 colorectal cancer cell lines. Low dose of resveratrol reduced β-catenin expression, which can affect Wnt-signaling and suppress the proliferation. On the other hand, resveratrol can decrease GSK-3β expression in low dose, but increase its expression in high doses. Therefore, resveratrol can act in two ways: First, by increasing Wnt-signaling pathway, it can play a role in the destruction of β-catenin complex and inhibition of accumulation in the cytoplasm which finally leads to transcription/activation of Wnt target genes that induce cellular proliferation. Second, resveratrol via decreasing GSK-3β affects some transcriptional or molecular factors in cell cycle and results in proliferation inhibition and apoptosis induction. Moreover, According to the phosphorylation of gene products and their effects on different cellular signalings, assessing the amount of protein produced by the western blotting is recommended in future studies.

ACKNOWLEDGMENTS:

This research has been supported by Tehran University of Medical Sciences and Health Services grant number 92-03-161-24438.

REFERENCES:

1. Parkin DM PP, Ferlay J. Global cancer statistics. CA Cancer J Clin. 1999;49(2):33–64.
2. Center MM Ja, Smith R a., Ward E. Worldwide variations in colorectal cancer. Dis Colon Rectum 2010;53:1099.
3. Richard S. J CJY, and Eléonore B. Glycogen Synthase Kinase-3 (GSK3): Inflammation, Diseases, and Therapeutics. NIH Public Access 2012;29(6):997–1003.
4. E. G. Modifiable risk factors for colon cancer. Gastroenterol Clin North Am 2002;31(4):925–43.
5. Ferrari P JM, Norat T, Moskal A, Slimani N, Olsen A, et al. Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European Prospective Investigation into Cancer and Nutrition (EPIC). Int J Cancer 2007;121(9):2065-72.
6. Ji Q LX, Fu X, Zhang L, Sui H, Zhou L, et al. . Resveratrol inhibits invasion and metastasis of colorectal cancer cells via MALAT1 mediated Wnt/β-catenin signal pathway. . PLoS One 2013;8(11).
7. IJ. F. The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited. Nat Rev Cancer England. 2003;3(6):453–8.
8. Oving IM CH. Molecular causes of colon cancer. Eur J Clin Invest 2002;32(6):448–57.
9. MacDonald BT TK, He X. . Wnt/ β-catenin Signaling: Components, Mechanisms, and Diseases. Dev Cell Elsevier Inc. 2009;17(1):9–26.
10. Nusse R VH. Wnt genes. . Cell 1992;69(7):1073–87.
11. PJ. M. Beta-Catenin Signaling and Cancer. Bioessays 1999;21(12):1021–30.
12. Ding Y SS, Tang W, Zhang X, Chen S, Zhu G, et al. Enrichment of the β-catenin-TCF complex at the S and G2 phases ensures cell survival and cell cycle progression. J Cell Sci 2014;127(22):4833–45.
13. Bienz M CH. Linking Colorectal Cancer to Wnt Signaling Review. cell press 2000;103:311–20.
14. Kishida M KS, Kishida S, Matsubara K, Nakashima S. Axin prevents Wnt-3a-induced accumulation of b -catenin. Oncogene. 1999;18:979-85.
15. J. L. Glycogen synthase kinase 3β (GSK-3β) in tumorigenesis and cancer chemotherapy. Cancer Lett 2009;273(2):194–200.
16. Liu Y-Z WK, Huang J, Liu Y, Wang X, Meng Z-J, et al. The PTEN/PI3K/Akt and Wnt/β-catenin signaling pathways are involved in the inhibitory effect of resveratrol on human colon cancer cell proliferation. Int J Oncol. 2014:104–12.
17. Preet R MP, Satapathy SR, Kundu CN. 1, 3- bis(2-chloroethyl)-1-nitrosourea enhances the inhibitory effect of Resveratrol on 5-fluorouracil sensitive/resistant colon cancer cells. World J Gastroenterol 2013;19(42):7374–88.
18. Miki H UN, Kimura A, Sasaki T, Yuri T, Yoshizawa K, et al. Resveratrol induces apoptosis via ROS-triggered autophagy in human colon cancer cells. Int J Oncol 2012;40(4):1020–8.
19. Bhat KPL KJn, Pezzuto JM. BIOLOGICAL EFFECTS OF RESVERATROL. Antioxid Redox Signal. 2001;3(6):1041-64.
20. Kalra N RP, Prasad S, Shukla Y. Resveratrol induces apoptosis involving mitochondrial pathways in mouse skin tumorigenesis. Life Sci 2008;82(7-8):348–58.

21. Hope C PK, Planutiene M, Moyer MP, Karanjodh S, Woo J, et al. Low concentrations of resveratrol inhibit Wnt signal throughput in colon-derived cells : Implications for colon cancer prevention. Mol Nutr Food Res. 2008;52:52–61.
22. Chen H HL, Shia Y, Lin M, Lin C. The b -catenin / TCF complex as a novel target of resveratrol in the Wnt / b -catenin signaling pathway. . Biochem Pharmacol Elsevier Inc. 2012;84(9):1143–53.
23. H. Wang HZ, Y. Zou, Q. Liu, C. Guo, G. Gao, et al.,. Resveratrol modulates angiogenesis through the GSK3β/β-catenin/TCF-dependent pathway in human endothelial cells. Biochem Pharmacol. 2010;80:1386–95.
24. M.I. Patel AG, C.S. Dey,. Potentiation of neuronal insulin signaling and glucose uptake by resveratrol: The involvement of AMPK,. Pharmacol Reports 2011;63:1162–8.
25. Yabroff KR MA, Feuer E, Brown ML. Projections of the costs associated with colorectal cancer care in the United States, 2000-2020. Health Econ England. 2008;17(8):947–59.
26. SRaAP. M. Signaling in colon cancer stem cells. . Roy and Majumdar Journal of Molecular Signaling 2012;7(11).
27. Clevers H NR. Review Wnt / b -Catenin Signaling and Disease. Cell. 2012;149(6):1192-205.
28. Stambolic V WJ. Mitogen inactivation of glycogen synthase kinase-3 beta in intact cells via serine 9 phosphorylation. . Biochem J 1994;303(3):701–4.
29. Jope RS YÆ. Glycogen Synthase Kinase-3 (GSK3): Inflammation, Diseases , and Therapeutics. Neurochem Res. 2007;32(4-5):577–95.
30. Tenbaum SP O-MP, Puig I, Chicote I, Arqués O, Landolfi S, et al. β-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. Nat Med 2012;18(6):892–901.
31. Ito T AY, Tanaka T, Iinuma M and Nozawa Y. Vaticanol C,. a novel resveratrol tetramer, inhibits cell growth through induction of apoptosis in colon cancer cell lines. . Biol Pharm Bull. 2002;25:147-8.
32. Aggarwal BB BA, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of Resveratrol in Prevention and Therapy of Cancer : Preclinical and Clinical Studies. Anticancer Res. 2004;24(5A):2783-840
33. Masckauchan TNH SC, Funahashi Y, Li C-M, Kitajewski J. Wnt/beta-catenin signaling induces proliferation, survival and interleukin-8 in human endothelial cells. . Angiogenesis. 2005;8(1):43–51.
34. Rulifson IC KS, Heiser PW, Berge D, Chen H, Gu X, et al. . Wnt signaling regulates pancreatic b cell proliferation. Proc Natl Acad Sci U S A. 2007;104(15):6247–52.
35. Huang C MW, Goranson A and Dong Z. . Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. . Carcinogenesis. 1999;20:237- 42.
36. Zhou H SL, Li X, Zhang X, Gao G, Guo C, et al. . Resveratrol augments the canonical Wnt signaling pathway in promoting osteoblastic differentiation of multipotent mesenchymal cells. . Exp Cell Res 2009;315(17):2953–62.