Omentin inhibits the resistin-induced hypertrophy of H9c2 cardiomyoblasts by inhibiting the TLR4/MyD88/NF-κB signaling pathway

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Abstract. The recently identified adipocytokine omentin was previously found to be expressed mainly in human omental and visceral adipose tissues. As such, reduced plasma concentrations of omentin were revealed to be associated with increased risks of cardiovascular diseases. Omentin has also been previously demonstrated to exert anti-inflammatory effects. By contrast, resistin is a protein that has been associated with obesity and type-2 diabetes mellitus, and the serum concentration of resistin is increased significantly in these populations. Resistin is involved in mediating inflammation development, where they can promote cardiac hypertrophy in humans through toll-like receptor 4 (TLR4)-related signaling. In the present study, the potential effects of omentin on resistin-induced hypertrophy in H9c2 cardiomyoblasts were investigated. In the absence/presence of omentin, H9c2 cardiomyoblasts were treated with resistin. Omentin was found to significantly inhibit resistin-induced increases in the surface area of H9c2 cardiomyoblasts as determined by immunofluorescence. In addition, omentin significantly inhibited resistin-induced increases in the mRNA expression of atrial natriuretic factor, brain natriuretic peptide, β-myosin heavy chain (which is a characteristic feature of cardiac hypertrophy) and TLR4, which was determined using reverse-transcription-quantitative PCR. According to western blotting results, omentin significantly inhibited resistin-induced ERK phosphorylation, which is an important mediator of cardiomyoblast hypertrophy. Furthermore, omentin significantly inhibited resistin-induced protein expression of TLR4, myeloid differentiation factor 88 (MyD88) and NF-κB phosphorylation, both of which are important members of inflammatory signaling. To conclude, data from the present study suggest that omentin can inhibit resistin-induced H9c2 cardiomyoblast hypertrophy through inhibition of the TLR4/MyD88/NF-κB signaling pathway. Therefore, omentin serve as an attractive therapeutic target against resistin-induced cardiac hypertrophy.

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Introduction

It has been previously reported that ~66% of the mortality cases associated with diabetes mellitus (DM) can be attributed to cardiovascular diseases (1). In the absence of changes in blood pressure and coronary artery disease, DM can alter the structure and function of the heart by causing a condition known as 'diabetic cardiomyopathy' (2). Initially, diabetic cardiomyopathy is characterized by cardiac muscle hypertrophy and associated diastolic dysfunction, which is typically followed by systolic dysfunction and ultimately heart failure (3). A maladaptive inflammatory response has been implicated in the occurrence of cardiac hypertrophy during diabetic cardiomyopathy (4). Previous studies have revealed that alleviating the inflammatory response during cardiac hypertrophy is beneficial to the survival of diabetic cardiomyopathy (5,6).

The toll-like receptor 4 (TLR4)/NF-κB signaling pathway serves a key role in cardiac hypertrophy (7). Activity of this pathway was previously found to be significantly promoted in the hypertrophic myocardium (8). Gao *et al* (9) previously reported that inhibition or knockdown of TLR4/myeloid differentiation primary response 88 (MyD88) signaling pathway attenuated inflammatory and hypertrophic responses in transverse aortic constriction or angiotensin-II infusion of mice and cardiomyocytes isolated from mouse neonatal ventricles.

Resistin is a cysteine-rich polypeptide that is mainly secreted by macrophages in humans and by adipose tissues in rodents and humans (10). It has been reported to be positively

associated with obesity and the development of type-2 diabetes mellitus (T2DM) (10). In addition, serum levels of resistin were significantly higher in obese and T2DM patients compared with those in healthy subjects (11,12). Resistin has been previously found to be involved in mediating inflammation, insulin resistance, cardiac hypertrophy, hypertension, atherosclerosis, coronary artery disease and rheumatic diseases (13-17). Kim *et al* (14) previously found that resistin overexpression could decrease myocardial contractility, in addition to endowing primary cardiomyoblasts with hypertrophic phenotypes to promote cardiac hypertrophy. Other studies have also found that resistin can induce inflammation, insulin resistance and hypertension through a TLR4-dependent signaling pathway (13,15).

Omentin, also known as intelectin-1, is a cytokine that is typically secreted by the adipose tissue (adipocytokine). It is mainly expressed in omental and visceral adipose tissues in humans (18). Physiologically, omentin has been found to exhibit various pharmacological effects in the cardiovascular system, with protective effects against vascular inflammation (19), atherogenesis (20) and myocardial ischemia (21) among those reported. In addition, reduced circulating levels of omentin have been associated with increased risk of obesity-related diseases, including metabolic syndrome and T2DM (22). Previous clinical studies have revealed that decreased plasma concentrations of omentin are associated with increased incidences of atherosclerosis and ischemic heart disease (23,24). Therefore, these previous findings of omentin aforementioned suggest that it may serve a protective role against cardiovascular disorders associated with metabolsim. However, the mechanistic role of omentin in resistin-induced cardiac hypertrophy remains poorly understood.

In the present study, the potential effects of omentin on resistin-induced hypertrophy in H9c2 cardiomyoblasts were investigated. The present study will investigate the relationship of omentin, resistin and TLR4/MyD88/NF-κB pathway through H9c2 cardiomyoblasts related experiments.

Materials and methods

Materials. Recombinant human omentin protein (cat. no. RD172100025) was purchased from BioVendor. Recombinant human resistin protein (450-19-25) was obtained from PeproTech, Inc. Anti-TLR4 antibody (cat. no. ab95562) was purchased from Abcam. Antibodies against MyD88 (cat. no. 4283), NF-κB p65 (cat. no. 8242), phosphorylated (p)-NF-κB p65 (cat. no. 3033), β-tubulin (cat. no. 2146), p-ERK (Thr-202/Tyr-204; cat. no. 4370), and ERK (cat. no. 4695) were obtained from Cell Signaling Technology, Inc. Antibody against β-actin (cat. no. SAB3500350) was from Sigma-Aldrich; Merck KGaA.

Culture of H9c2 cardiomyoblasts. H9c2 rat cardiomyoblasts were obtained from American Type Culture Collection. H9c2 cells were cultured in DMEM (Wako Pure Chemical Industries, Ltd.) containing 10% FBS (Zhejiang Tianhang Biotechnology, Co., Ltd.) and 1% penicillin-streptomycin in an atmosphere of 5% CO_2 at 37˚C, consistent with protocols described in a previous study (25). After the H9c2 cardiomyoblasts reached 90% confluence, they were growth-arrested in FBS-free medium for 24 h before stimulation with omentin or resistin at 37˚C.

Immunofluorescence to ascertain the surface area of H9C2 cardiomyoblasts. After the H9c2 cardiomyoblasts were cultured in serum-free medium for 24 h, they were treated with resistin (100 ng/ml) for 48 h with or without omentin (3, 30 and 300 ng/ml; 1 h) pre-treatment (26-28) at 37˚C. To determine the extent of α-actin organization within sarcomeres, cultured H9c2 cardiomyoblasts were fixed in 100% methanol for 10 min at 4˚C, washed with PBS three times and blocked with 10% normal goat serum (Shanghai Yisheng Biotechnology, Co., Ltd.) for 30 min at room temperature. The cardiomyoblasts were then incubated with the mouse anti-α-sarcomeric actin primary monoclonal antibody (1:500; cat. no. 113200; Sigma-Aldrich; Merck KGaA) for 1 h at room temperature, followed by incubation with the FITC-conjugated secondary antibody (1:1,000; cat. no. sc-2359; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature (29). The nuclei of the H9c2 cardiomyoblasts were stained using Hoechst 33258 (1 μg/ml; Sigma-Aldrich; Merck KGaA) for visualization for 1 h at 37˚C. A fluorescence microscope (Carl Zeiss, AG) was used to image the samples. The surface area of H9c2 cardiomyoblasts was measured using ImageJ 1.49 (National Institutes of Health) from two-dimensional images of 50 cells selected at random in 20 fields at x400 magnification.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the H9c2 cardiomyoblasts using TRIzol® Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to manufacturer protocols. The RNA concentration was measured using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Inc.). Reverse transcription was performed using a complementary DNA (cDNA) reverse transcription kit PrimeScript RT Master Mix (cat. no. RR036Q; Takara Bio, Inc.). The conditions of reaction were: 37˚C for 15, 85˚C for 5 sec. The obtained cDNA was then subjected to qPCR for measurement of mRNA expression of atrial natriuretic peptide (ANF), B-type natriuretic peptide (BNP), β-myosin heavy chain (β-MHC) and TLR4 using a TB Green Premix Ex Taq II (cat. no. RR820Q; Takara Bio, Inc.). All reactions were performed using the a Applied Biosciences 7500 system (Thermo Fisher Scientific, Inc.). Standard procedure for two-step PCR amplification: Stage 1, pre-degeneration, 1 cycle, 95˚C for 30 sec; stage 2, PCR reaction, 40 cycles, 95˚C for 3 sec, 60˚C for 30 sec. β-actin was used as the internal reference. Primer sequences were procured from Sangon Biotech Co., Ltd. The primer sequences were as follows: ANF forward, 5'-AGGCCA TATTGGAGCAAATC-3' and reverse, 5'-CATCTTCTC CTCCAGGTGGT-3'; BNP forward, 5'-GTGCTGCCCCAG ATGATTCT-3' and reverse, 5'-GCAGCTTCTGCATCG TGGAT-3'; β-MHC forward, 5'-TGCTCTACAATCTCA AGGAGAGGT-3' and reverse, 5'-TGTTGACGGTCTTAC CAGCTC-3'; TLR4 forward, 5'-AAGTTATTGTGGTGG TGTCTAG-3' and reverse, 5'-GAGGTAGGTGTTTCTGCT AAG-3' and β-actin forward, 5'-GAACCCTAAGGCCAA CCG-3' and reverse, 5'-TACGTACATGGCTGGGGTGT-3'. Relative quantification of mRNA expression was analyzed using the $2^{-\Delta\Delta Cq}$ method (30).

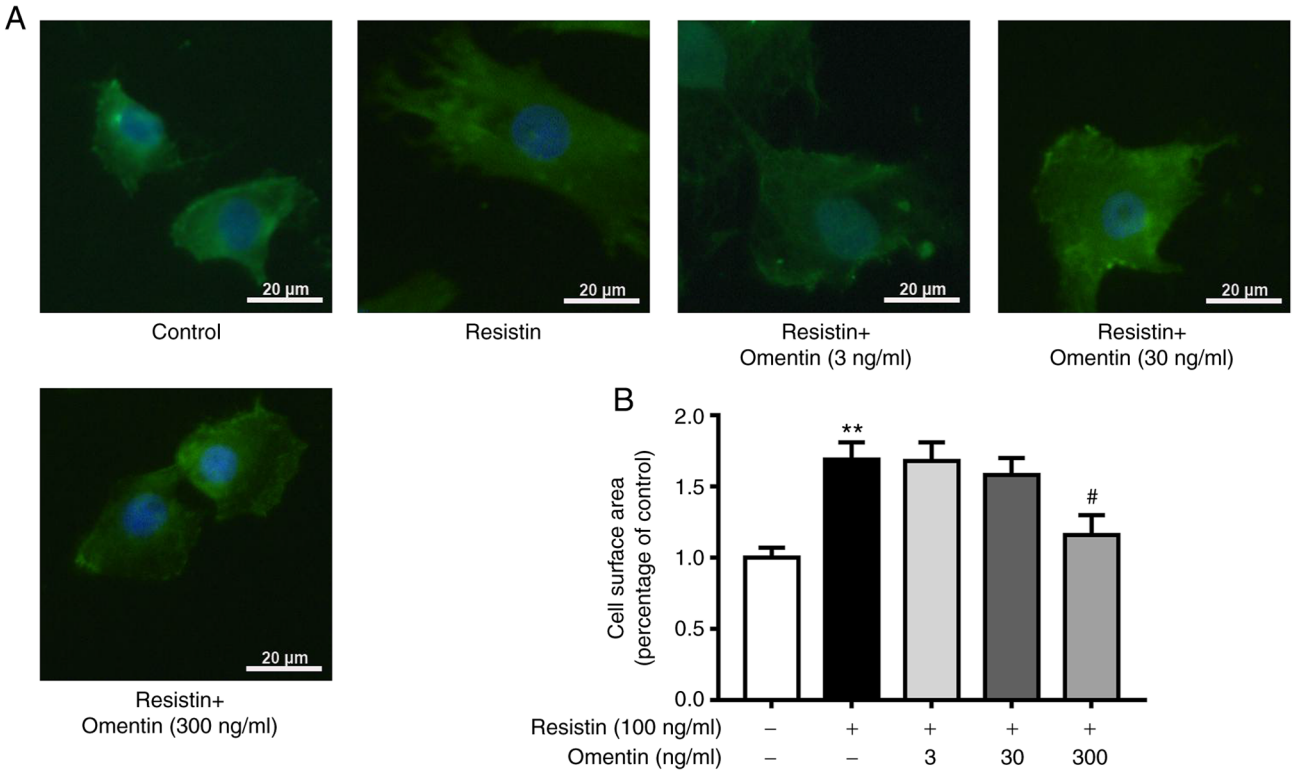

Figure 1. Effects of omentin on the surface area of resistin-induced H9c2 cardiomyoblasts. H9c2 cardiomyoblasts were treated with resistin (100 ng/ml, 48 h) in the absence or presence of omentin (3, 30 and 300 ng/ml, 1 h). (A) Representative two-dimensional images of 50 cells selected at random. n=6. The H9c2 cardiomyoblasts were stained by immunofluorescence with the α-sarcomeric actin antibody, which is shown as green fluorescence. Nuclei of H9c2 cardiomyoblasts were stained using Hoechst 33258, which are shown as blue fluorescence. (Scale bars, 20 μm). (B) The surface area of cardiomyoblasts as measured by ImageJ 1.49. **P<0.01 vs. Control; $^{\#}$P<0.05 vs. Resistin-only.

Western blotting. After the H9c2 cardiomyoblasts were treated with resistin (100 ng/ml) for 1 or 12 h with or without pretreatment with omentin (300 ng/ml; 1 h) at 37˚C, samples of total protein were obtained by homogenizing H9c2 cardiomyoblasts with RIPA lysis buffer (CoWin Biosciences). Protein concentration was determined using the bicinchoninic acid assay before 5X Laemmli buffer was added. An equal amount of protein (25-30 μg) was separated by 10% SDS-PAGE, before the proteins were transferred onto PVDF membranes (MilliporeSigma). After blockade with 0.5% non-fat milk or 5% bovine serum albumin (Boster Biological Technology) for 2 h at room temperature, the PVDF membranes were incubated with primary antibodies [TLR4, MyD88, phosphorylated (p-)-NF-κB p65, NF-κB p65, p-ERK and ERK antibodies at 1:1,000 dilution; β-tubulin and β-actin at 1:2,000 dilution] overnight at 4˚C. After washing three times with TBS containing 0.1% Tween 20, the PVDF membranes were incubated with HRP-conjugated secondary antibodies (1:2,000 dilution; cat. no. 70745; Cell Signaling Technology, Inc.) for 1 h at room temperature. Immunoreactive bands were visualized using an enhanced chemiluminescence detection system (Bio-Techne) and the densitometry was quantified by ImageJ 1.49 (National Institutes of Health).

Statistical analysis. Data are presented as the mean ± SEM at least 4 independent experiments. Statistical comparisons were performed using one-way ANOVA followed by Bonferroni's test. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were conducted using Graphpad Prism 5 (GraphPad Software, Inc.).

Results

Effects of omentin on the surface area of resistin-induced H9c2 cardiomyoblasts. Resistin has been previously shown to induce cardiomyoblast hypertrophy (14). In the present study, it was observed that resistin (100 ng/ml) significantly increased the surface area of the H9c2 cardiomyoblasts (1.69±0.12-fold relative to control; P<0.01; Fig. 1). Therefore, the potential effects of omentin (3, 30 and 300 ng/ml, 1 h) pre-treatment on this increase in the surface area of resistin-induced H9c2 cardiomyoblasts were examined. Omentin (300 ng/ml, 1 h) was found to significantly reverse the resistin (100 ng/ml, 48 h)-induced increases in the surface area of H9c2 cardiomyoblasts (300 ng/ml omentin + resistin, 1.16±0.14-fold relative to control; P<0.05; Fig. 1).

Effects of omentin on the mRNA expression of ANF, BNP, β-MHC and TLR4 in resistin-induced H9c2 cardiomyoblasts. Re-activation of fetal genes ANF, BNP and β-MHC is a characteristic feature of cardiac hypertrophy (31). Therefore, the present study next assessed the potential effects of omentin (300 ng/ml, 1 h) on the mRNA expression of ANF, BNP and β-MHC in resistin-induced H9c2 cardiomyoblasts. In addition, the effect of omentin (300 ng/ml; 1 h) on resistin-induced mRNA expression of TLR4 was also assessed in H9c2 cardiomyoblasts. Omentin

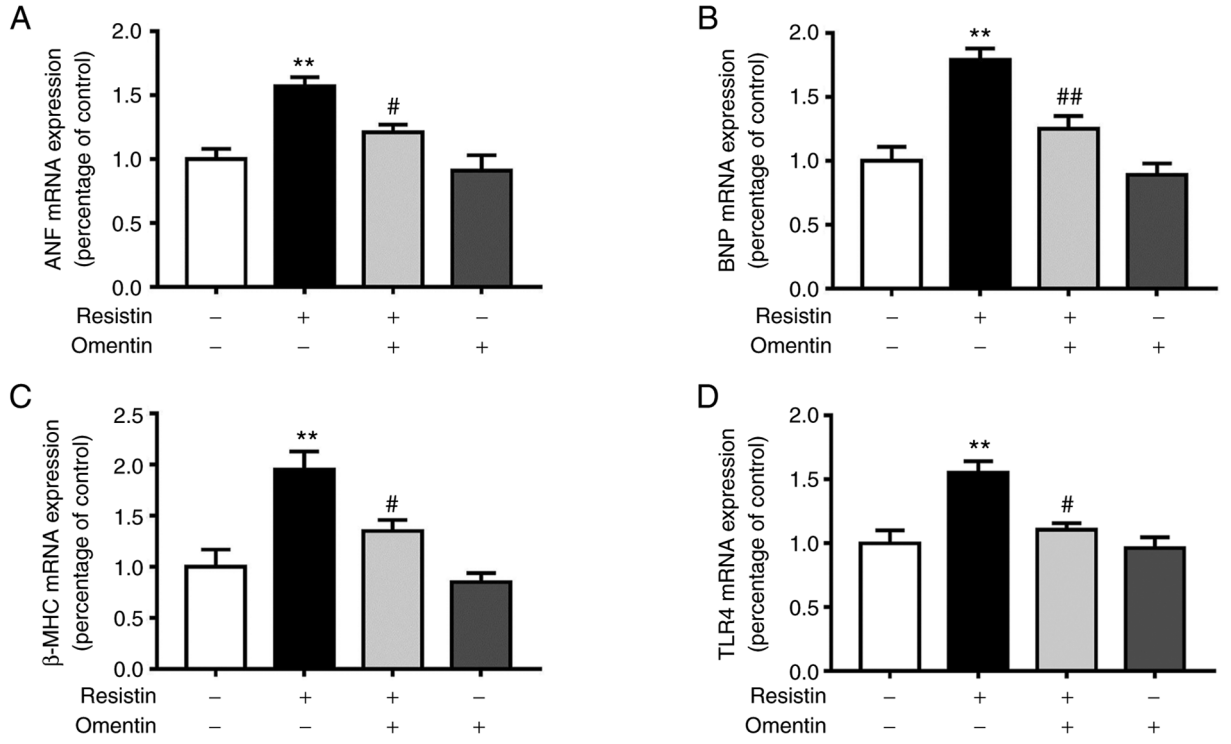

Figure 2. Effects of omentin on the mRNA expression of ANF, BNP, β-MHC and TLR4 in resistin-induced H9c2 cardiomyoblasts. After the H9c2 cardiomyoblasts were treated with resistin (100 ng/ml, 24 h) in the absence or presence of omentin (300 ng/ml, 1 h), total RNA was then extracted. (A) Expression of ANF, (B) BNP, (C) β-MHC and (D) TLR4 mRNA was measured by reverse transcription-quantitative PCR. n=4. **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. resistin-only. ANF, atrial natriuretic peptide; BNP, B-type natriuretic peptide; β-MHC, β-myosin heavy chain; TLR4, toll-like receptor 4.

significantly reversed the resistin-induced (100 ng/ml, 24 h) increase in expression of ANF mRNA (resistin, 1.57±0.07-fold relative to control; omentin + resistin, 1.21±0.06-fold relative to control; P<0.05; Fig. 2A). Omentin also significantly reversed the resistin-induced (100 ng/ml, 24 h) increase in expression of BNP mRNA (resistin, 1.79±0.09-fold relative to control; omentin + resistin, 1.25±0.10-fold relative to control; P<0.01; Fig. 2B). In addition, omentin significantly reversed the resistin-induced (100 ng/ml, 24 h) increase in expression of β-MHC mRNA (resistin, 1.95±0.18-fold relative to control; omentin + resistin, 1.35±0.11-fold relative to control; P<0.05; Fig. 2C). Omentin significantly reversed the resistin-induced (100 ng/ml, 24 h) increase in expression of TLR4 mRNA (resistin, 1.55±0.09-fold relative to control; omentin + resistin, 1.11±0.05-fold relative to control; P<0.05; Fig. 2D).

Effects of omentin on ERK phosphorylation in resistin-induced H9c2 cardiomyoblasts. An important process in cardiomyoblast hypertrophy is ERK activation (28,29). The present study next investigated the effects of omentin (300 ng/ml, 1 h) on resistin-induced ERK phosphorylation in H9c2 cardiomyoblasts by western blotting. Stimulation of H9c2 cardiomyoblasts with resistin (100 ng/ml, 1 h) had no effects on the protein expression of total ERK, but significantly increased phosphorylation levels of ERK at Thr-202/Tyr-204 and the p-ERK/ERK ratio (Fig. 3), suggesting an increase in ERK activity due to resistin. However, omentin pre-treatment significantly prevented the resistin-induced ERK phosphorylation (resistin, 2.48±0.19-fold relative to control; omentin + resistin; 1.64±0.20-fold relative to control; P<0.05; Fig. 3).

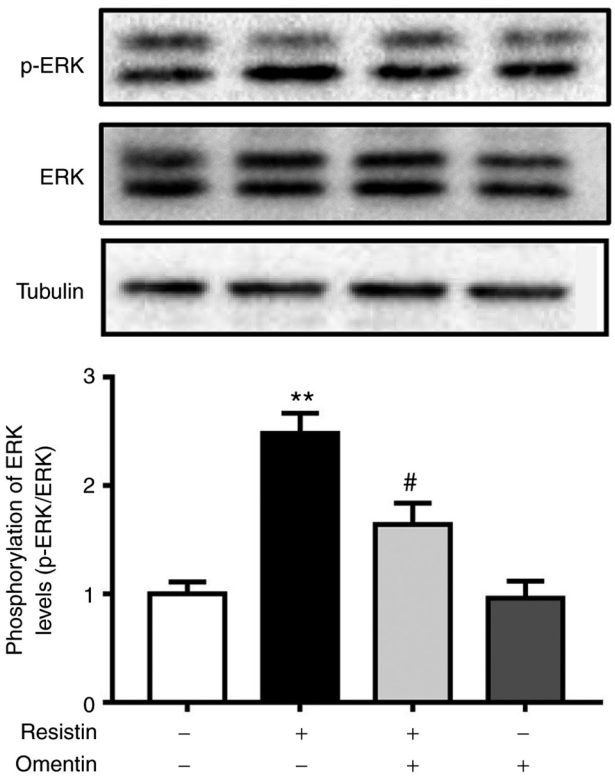

Figure 3. Effects of omentin on ERK activation in resistin-induced H9c2 cardiomyoblasts. After the H9c2 cardiomyoblasts were treated with resistin (100 ng/ml, 1 h) in the absence or presence of omentin (300 ng/ml, 1 h), cardiomyoblast protein lysates were obtained. Phosphorylation of ERK was measured by western blotting. n=4. **P<0.01 vs. Control; $^{\#}$P<0.05 vs. Resistin-only. p-, phosphorylated.

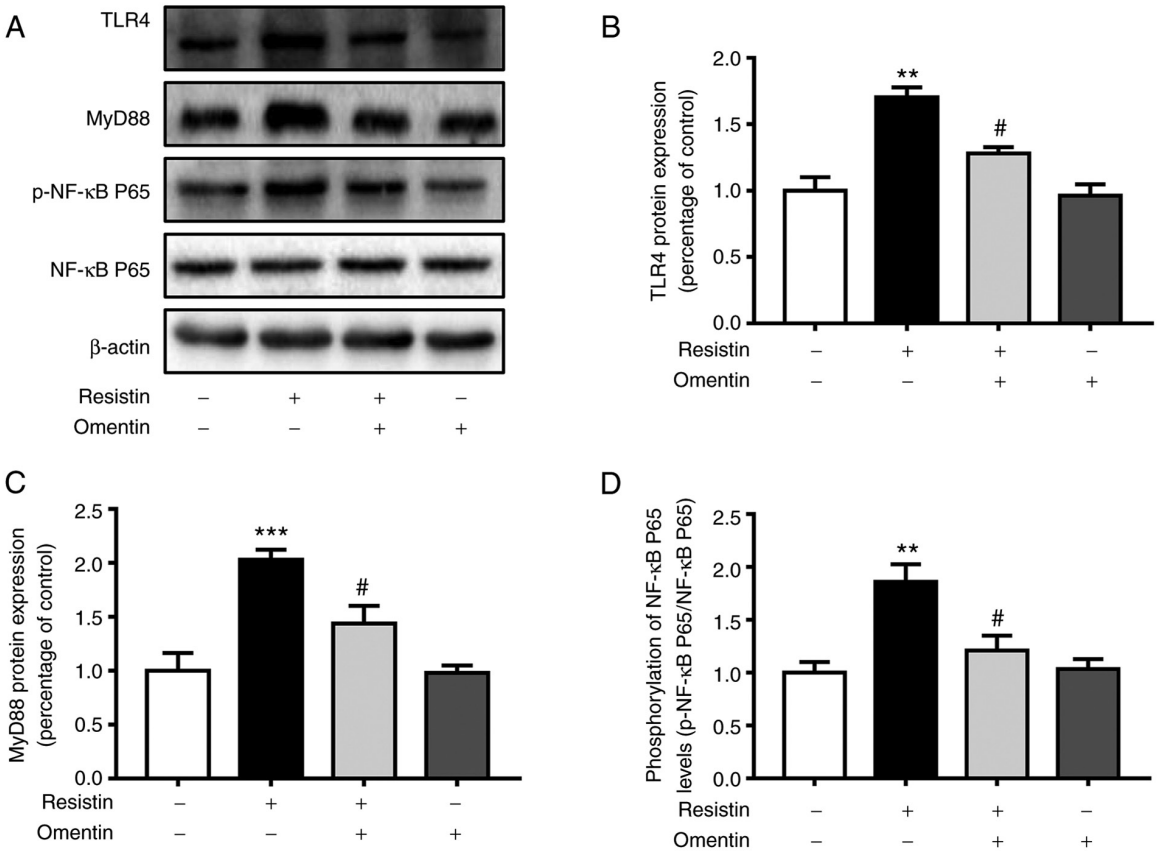

Figure 4. Effects of omentin on the expression of TLR4, MyD88 and activation of NF-κB p65 in resistin-induced H9c2 cardiomyoblasts. H9c2 cardiomyoblasts were treated with resistin (100 ng/ml, 12 h) in the absence or presence of omentin (300 ng/ml, 1 h). (A) Protein levels of TLR4, MyD88, p-NF-κB p65 and NF-κB p65 were measured by western blotting. n=4. (B) Protein expression of TLR4, (C) MyD88 and (D) Phosphorylation of NF-κB p65 was quantified by western blotting. n=4. **P<0.01 and ***P<0.001 vs. control; $^{\#}$P<0.05 vs. resistin-only. TLR4, toll-like receptors; MyD88, myeloid differentiation factor 88; p-, phosphorylated.

Effect of omentin on the protein expression of TLR4, MyD88 and NF-κB p65 phosphorylation in resistin-induced H9c2 cardiomyoblasts. The present study next assessed the effects of omentin (300 ng/ml, 1 h) on the resistin-induced protein levels of TLR4, MyD88, p-NF-κB p65 and NF-κB p65 in H9c2 cardiomyoblasts by western blotting. Omentin significantly inhibited the resistin-induced (100 ng/ml, 12 h) protein expression of TLR4 (resistin, 1.70±0.07-fold relative to control; omentin + resistin, 1.28±0.09-fold relative to control, P<0.05; Fig. 4B) in H9c2 cardiomyoblasts. Omentin also significantly prevented the resistin-induced (100 ng/ml, 12 h) expression of the MyD88 protein (resistin, 2.03±0.09-fold relative to control; omentin + resistin, 1.44±0.17-fold relative to control, P<0.05; Fig. 4C) in H9c2 cardiomyoblasts. In addition, omentin significantly reversed the resistin-induced (100 ng/ml, 12 h) protein phosphorylation of NF-κB p65 (resistin, 1.86±0.17-fold relative to control; omentin + resistin, 1.21±0.14-fold relative to control, P<0.05; Fig. 4D) in H9c2 cardiomyoblasts. However, omentin had no effects on the protein expression of total NF-κB p65, suggesting that omentin inhibited the activity of resistin-induced TLR4/MyD88/NF-κB signaling in H9c2 cardiomyoblasts.

Discussion

In the present study, it was demonstrated that omentin inhibited the resistin-induced hypertrophy of H9c2 cardiomyoblasts. In addition, omentin inhibited resistin-induced expression of TLR4, MyD88 and NF-κB p65 phosphorylation, which are important molecular components of the TLR4/MyD88/NF-κB inflammatory pathway (32). Omentin also inhibited the resistin-induced re-activation of the expression of fetal genes ANF, BNP and β-MHC, which is the characteristic feature of cardiac hypertrophy (31). Furthermore, it was demonstrated that omentin inhibited the resistin-induced activation of ERK, which is an important mediator of cardiomyoblast hypertrophy (33,34). Taken together, these observations suggest that omentin can inhibit the hypertrophy of resistin-induced H9c2 cardiomyoblasts by blunting the activity of TLR4/MyD88/NF-κB signaling.

TLRs are pathogen pattern-recognition receptors and serve as important components of the innate immune system (35). The first member of the TLR family to be identified was TLR4 (32), which can mediate myocardial inflammation (36). In addition, the TLR4-activated mediation of inflammatory signaling serves an important role in myocarditis, cardiac hypertrophy, ischemia-reperfusion injury and myocardial infarction (37-39). A number of studies have previously shown that production of proinflammatory factors is regulated by the TLR4/MyD88/NF-κB signaling pathway, which in turn induces inflammation in the myocardial tissues and causing injury (40,41). In addition, it has been reported that resistin is involved in myocardial inflammation through

a TLR4-associated pathway to cause myocardial tissue injury (13-15,42,43).

Cardiac hypertrophy is an adaptive change of the myocardium to increase the volume or pressure loads (44). However, this physiological adaptation is frequently accompanied with pathological changes (31). Activation of several intracellular signaling pathways is closely associated with the occurrence and development of cardiac hypertrophy. Han *et al* (45) found TLR4 activation could initiate myocardial remodeling. In another study, Ehrentraut *et al* (46) previously found that TLR4 antagonists could reduce cardiac hypertrophy in mice. Activation of the TLR4/MyD88/NF-κB signaling pathway has been demonstrated to increase the expression of a number of the proinflammatory cytokines, such as TNF-α and IL-6, which involved in the inflammatory response to cause myocardial injury (40,41). In addition, cardiac hypertrophy and myocardial inflammation were stifled effectively by inhibiting the activity of the TLR4 signaling pathway (46,47). In particular, inhibiting the expression of NF-κB p65 which is one of components in the NF-κB signaling pathway was able to reduce the myocardial inflammatory response, inhibit the development of cardiac hypertrophy and reduce the risk of heart failure in the transgenic mouse (48). The present study demonstrated that resistin could significantly activate the TLR4/MyD88/NF-κB signaling pathway in H9c2 cardiomyoblasts, to induce an increase in the surface area of H9c2 cardiomyoblasts.

Omentin is a newly identified adipocytokine that was shown to exert an anti-inflammatory effect (49,50). Previous studies have demonstrated that various cardiovascular diseases, including carotid atherosclerosis and coronary artery disease, manifest with reduced plasma concentrations of omentin (23,24,51). Genre *et al* (52) found that low serum omentin levels were associated with cardiovascular risk factors, including obesity and high atherosclerosis indices, in patients with axial spondyloarthritis. In addition, a number of previous studies have demonstrated high plasma concentrations of omentin to be associated with superior outcomes in patients with acute heart failure or coronary heart disease (49,53). Ma *et al* (8) found that inhibition of TLR4 pathway can inhibit myocardial hypertrophy in mice. In the present study, H9c2 cardiomyoblasts were first pre-treated with omentin (3, 30 and 300 ng/ml) and then found that 300 ng/ml omentin prevented the resistin-induced hypertrophy of H9c2 cardiomyoblasts. However, omentin at 3 and 30 ng/ml could not, which are consistent with the cardiovascular benefits of increasing the human serum omentin concentration (53). In addition, it was found that omentin inhibited the mRNA and protein expression of TLR4 induced by resistin in H9c2 cardiomyoblasts. Omentin also inhibited the protein expression of MyD88 and phosphorylation of NF-κB p65 after resistin stimulation in H9c2 cardiomyoblasts. Therefore, the ability of omentin to attenuate cardiomyoblast hypertrophy is likely due to its ability to inhibit the expression of components in the TLR4/MyD88/NF-κB signaling pathway in H9c2 cardiomyoblasts. Collectively, these data suggest that omentin-mediated inhibition of the TLR4/MyD88/NF-κB signaling pathway may represent a common pathway that leads to the beneficial actions of omentin in the cardiovascular system.

ERK activation is an important process in cardiomyoblast hypertrophy (33,34). Inhibition of TLR4 was shown to reduce ERK activity in various cell types, including cardiomyoblasts (44,45). This suggests that inhibition of TLR4 may reduce cardiomyoblasts hypertrophy. The present study demonstrated that stimulation of H9c2 cardiomyoblasts with resistin led to increased ERK activity. However, pre-treatment of the H9c2 cardiomyoblasts with omentin inhibited ERK activation in response to resistin treatment.

Reactivation of fetal myocardial genes, including ANF, BNP and β-MHC, is a characteristic feature of cardiac hypertrophy (31). The present study found that resistin could enhance the expression of these fetal myocardial genes in H9c2 cardiomyoblasts, whilst omentin could inhibit this resistin-induced expression of myocardial fetal genes in H9c2 cardiomyoblasts. Therefore, inhibition of resistin-induced cardiomyoblast hypertrophy by omentin may be associated with the inhibition of the expression of myocardial fetal genes.

A number of limitations are associated with the present study. The findings from the present study would require verification *in vivo*. In addition, agonists of the TLR4/MyD88/NF-κB signaling pathway or TLR4 knockdown would need be used to clarify if the inhibition of omentin on resistin-induced hypertrophy of cardiomyoblasts can be reversed. The extent of p65 activation in the nucleus would also require further confirmation in subsequent studies. Furthermore, measurements of the expression of inflammatory factors following omentin treatment will need to be performed. It would also be of interest to investigate the potential effects of omentin on resistin-induced oxidative stress (27).

To conclude, the present study showed that omentin can inhibit resistin-induced hypertrophy of H9c2 cardiomyoblasts through inhibition of the TLR4/MyD88/NF-κB signaling pathway. These results suggest that omentin may be an attractive therapeutic target against resistin-induced cardiac hypertrophy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YD designed the current study. YD and XY wrote the manuscript. XY established the hypertrophic model of H9c2 cardiomyoblasts. XY and MG performed H9c2 cardiomyoblasts immunofluorescence staining. XY and JY performed RT-qPCR. LW and PY performed western blotting. XY performed statistical analysis. YD and XY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Low Wang CC, Hess CN, Hiatt WR and Goldfine AB: Clinical update: Cardiovascular disease in diabetes mellitus: Atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus-mechanisms, management, and clinical considerations. Circulation 133: 2459-2502, 2016.
2. Boudina S and Abel ED: Diabetic cardiomyopathy revisited. Circulation 115: 3213-3223, 2007.
3. Jia G, Hill MA and Sowers JR: Diabetic cardiomyopathy: An update of mechanisms contributing to this clinical entity. Circ Res 122: 624-638, 2018.
4. Jia G, DeMarco VG and Sowers JR: Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. Nat Rev Endocrinol 12: 144-153, 2016.
5. Di Luigi L, Corinaldesi C, Colletti M, Scolletta S, Antinozzi C, Vannelli GB, Giannetta E, Gianfrilli D, Isidori AM, Migliaccio S, *et al*: Phosphodiesterase type 5 inhibitor sildenafil decreases the proinflammatory chemokine CXCL10 in human cardiomyocytes and in subjects with diabetic cardiomyopathy. Inflammation 39: 1238-1252, 2016.
6. Tan Y, Zhang Z, Zheng C, Wintergerst KA, Keller BB and Cai L: Mechanisms of diabetic cardiomyopathy and potential therapeutic strategies: Preclinical and clinical evidence. Nat Rev Cardiol 17: 585-607, 2020.
7. Xiao Z, Kong B, Yang H, Dai C, Fang J, Qin T and Huang H: Key player in cardiac hypertrophy, emphasizing the role of toll-like receptor 4. Front Cardiovasc Med 7: 579036, 2020.
8. Ma D, Zhang J, Zhang Y, Zhang X, Han X, Song T, Zhang Y and Chu L: Inhibition of myocardial hypertrophy by magnesium isoglycyrrhizinate through the TLR4/NF-κB signaling pathway in mice. Int Immunopharmacol 55: 237-244, 2018.
9. Gao W, Wang H, Zhang L, Cao Y, Bao JZ, Liu ZX, Wang LS, Yang Q and Lu X: Retinol-binding protein 4 induces cardiomyocyte hypertrophy by activating TLR4/MyD88 pathway. Endocrinology 157: 2282-2293, 2016.
10. Jamaluddin MS, Weakley SM, Yao Q and Chen C: Resistin: Functional roles and therapeutic considerations for cardiovascular disease. Br J Pharmacol 165: 622-632, 2012.
11. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS and Lazar MA: The hormone resistin links obesity to diabetes. Nature 409: 307-312, 2001.
12. Gerber M, Boettner A, Seidel B, Lammert A, Bär J, Schuster E, Thiery J, Kiess W and Kratzsch J: Serum resistin levels of obese and lean children and adolescents: Biochemical analysis and clinical relevance. J Clin Endocrinol Metab 90: 4503-4509, 2005.
13. Pine GM, Batugedara HM and Nair MG: Here, there and everywhere: Resistin-like molecules in infection, inflammation, and metabolic disorders. Cytokine 110: 442-451, 2018.
14. Kim M, Oh JK, Sakata S, Liang I, Park W, Hajjar RJ and Lebeche D: Role of resistin in cardiac contractility and hypertrophy. J Mol Cell Cardiol 45: 270-280, 2008.
15. Jiang Y, Lu L, Hu Y, Li Q, An C, Yu X, Shu L, Chen A, Niu C, Zhou L and Yang Z: Resistin induces hypertension and insulin resistance in mice via a TLR4-dependent pathway. Sci Rep 6: 22193, 2016.
16. Ohmori R, Momiyama Y, Kato R, Taniguchi H, Ogura M, Ayaori M, Nakamura H and Ohsuzu F: Associations between serum resistin levels and insulin resistance, inflammation, and coronary artery disease. J Am Coll Cardiol 46: 379-380, 2005.
17. Filková M, Haluzík M, Gay S and Senolt L: The role of resistin as a regulator of inflammation: Implications for various human pathologies. Clin Immunol 133: 157-170, 2009.
18. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, Shuldiner AR, Fried SK, McLenithan JC and Gong DW: Identification of omentin as a novel depot-specific adipokine in human adipose tissue: Possible role in modulating insulin action. Am J Physiol Endocrinol Metab 290: E1253-E1261, 2006.
19. Yamawaki H, Kuramoto J, Kameshima S, Usui T, Okada M and Hara Y: Omentin, a novel adipocytokine inhibits TNF-induced vascular inflammation in human endothelial cells. Biochem Biophys Res Commun 408: 339-343, 2011.
20. Watanabe K, Watanabe R, Konii H, Shirai R, Sato K, Matsuyama TA, Ishibashi-Ueda H, Koba S, Kobayashi Y, Hirano T and Watanabe T: Counteractive effects of omentin-1 against atherogenesis†. Cardiovasc Res 110: 118-128, 2016.
21. Kataoka Y, Shibata R, Ohashi K, Kambara T, Enomoto T, Uemura Y, Ogura Y, Yuasa D, Matsuo K, Nagata T, *et al*: Omentin prevents myocardial ischemic injury through AMP-activated protein kinase- and Akt-dependent mechanisms. J Am Coll Cardiol 63: 2722-2733, 2014.
22. Pan HY, Guo L and Li Q: Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. Diabetes Res Clin Pract 88: 29-33, 2010.
23. Shibata R, Takahashi R, Kataoka Y, Ohashi K, Ikeda N, Kihara S, Murohara T and Ouchi N: Association of a fat-derived plasma protein omentin with carotid artery intima-media thickness in apparently healthy men. Hypertens Res 34: 1309-1312, 2011.
24. Zhong X, Zhang HY, Tan H, Zhou Y, Liu FL, Chen FQ and Shang DY: Association of serum omentin-1 levels with coronary artery disease. Acta Pharmacol Sin 32: 873-878, 2011.
25. Luo JW, Zheng X, Cheng GC, Ye QH, Deng YZ and Wu L: Resistin-induced cardiomyocyte hypertrophy is inhibited by apelin through the inactivation of extracellular signal-regulated kinase signaling pathway in H9c2 embryonic rat cardiomyocytes. Biomed Rep 5: 473-478, 2016.
26. Ou HC, Lee WJ, Wu CM, Chen JF and Sheu WH: Aspirin prevents resistin-induced endothelial dysfunction by modulating AMPK, ROS, and Akt/eNOS signaling. J Vasc Surg 55: 1104-1115, 2012.
27. Cheleschi S, Gallo I, Barbarino M, Giannotti S, Mondanelli N, Giordano A, Tenti S, and Fioravanti1 A: MicroRNA mediate visfatin and resistin induction of oxidative stress in human osteoarthritic synovial fibroblasts Via NF-κB pathway. Int J Mol Sci 20: 5200, 2019.
28. Matsuo K, Shibata R, Ohashi K, Kambara T, Uemura Y, Hiramatsu-Ito M, Enomoto T, Yuasa D, Joki Y, Ito M, *et al*: Omentin functions to attenuate cardiac hypertrophic response. J Mol Cell Cardiol 79: 195-202, 2015.
29. Hardt SE, Tomita H, Katus HA and Sadoshima J: Phosphorylation of eukaryotic translation initiation factor 2Bepsilon by glycogen synthase kinase-3beta regulates beta-adrenergic cardiac myocyte hypertrophy. Circ Res 94: 926-935, 2004.
30. 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.
31. Sugden PH and Clerk A: Cellular mechanisms of cardiac hypertrophy. J Mol Med (Berl) 76: 725-746, 1998.
32. Akira S and Takeda K: Toll-like receptor signaling. Nat Rev Immunol 4: 499-511, 2004.
33. Yue TL, Gu JL, Wang C, Reith AD, Lee JC, Mirabile RC, Kreutz R, Wang Y, Maleeff B, Parsons AA and Ohlstein EH: Extracellular signal-regulated kinase plays an essential role in hypertrophic agonists, endothelin-1 and phenylephrine-induced cardiomyocyte hypertrophy. J Biol Chem 275: 37895-37901, 2000.
34. Tanaka K, Honda M and Takabatake T: Redox regulation of MAPK pathways and cardiac hypertrophy in adult rat cardiac myocyte. J Am Coll Cardiol 37: 676-685, 2001.

35. Lundberg AM, Ketelhuth DF, Johansson ME, Gerdes N, Liu S, Yamamoto M, Akira S and Hansson GK: Toll-like receptor 3 and 4 signalling through the TRIF and TRAM adaptors in haematopoietic cells promotes atherosclerosis. Cardiovasc Res 99: 364-373, 2013.
36. Molteni M, Gemma S, and Rossetti C: The role of toll-like receptor 4 in infectious and noninfectious inflammation. Mediators Inflamm 2016: 6978936, 2016.
37. Chimenti C, Verardo R, Scopelliti F, Grande C, Petrosillo N, Piselli P, De Paulis R and Frustaci A: Myocardial expression of Toll-like receptor 4 predicts the response to immunosuppressive therapy in patients with virus-negative chronic inflammatory cardiomyopathy. Eur J Heart Fail 19: 915-925, 2017.
38. Soraya H, Clanachan AS, Rameshrad M, Maleki-Dizaji N, Ghazi-Khansari M and Garjani A: Chronic treatment with metformin suppresses toll-like receptor 4 signaling and attenuates left ventricular dysfunction following myocardial infarction. Eur J Pharmacol 737: 77-84, 2014.
39. Lu M, Tang F, Zhang J, Luan A, Mei M, Xu C, Zhang S, Wang H and Maslov LN: Astragaloside IV attenuates injury caused by myocardial ischemia/reperfusion in rats via regulation of toll-like receptor 4/nuclear factor-κB signaling pathway. Phytother Res 29: 599-606, 2015.
40. Zhang J, Zhang J, Yu P, Chen M, Peng Q, Wang Z and Dong N: Remote ischaemic preconditioning and sevoflurane postconditioning synergistically protect rats from myocardial injury induced by ischemia and reperfusion partly via inhibition TLR4/MyD88/NF-κB signaling pathway. Cell Physiol Biochem 41: 22-32, 2017.
41. Ma SR and Xie XW: NLRC5 deficiency promotes myocardial damage induced by high fat diet in mice through activating TLR4/NF-κB. Biomed Pharmacother 91: 755-766, 2017.
42. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S and Ehtesham NZ: Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochem Biophys Res Commun 334: 1092-1101, 2005.
43. Tarkowski A, Bjersing J, Shestakov A and Bokarewa MI: Resistin competes with lipopolysaccharide for binding to toll-like receptor 4. J Cell Mol Med 14: 1419-1431, 2010.
44. Kang YJ: Cardiac hypertrophy: A risk factor for QT-prolongation and cardiac sudden death. Toxicol Pathol 34: 58-66, 2006.
45. Han J, Ye S, Zou C, Chen T, Wang J, Li J, Jiang L, Xu J, Huang W, Wang Y and Liang G: Angiotensin II causes biphasic STAT3 activation through TLR4 to initiate cardiac remodeling. Hypertension 72: 1301-1311, 2018.
46. Ehrentraut H, Weber C, Ehrentraut S, Schwederski M, Boehm O, Knuefermann P, Meyer R and Baumgarten G: The toll-like receptor 4-antagonist eritoran reduces murine cardiac hypertrophy. Eur J Heart Fail 13: 602-610, 2011.
47. Smeets PJ, Teunissen BE, Planavila A, de Vogel-van den Bosch H, Willemsen PH, van der Vusse GJ and van Bilsen M: Inflammatory pathways are activated during cardiomyocyte hypertrophy and attenuated by peroxisome proliferator-activated receptors PPARalpha and PPARdelta. J Biol Chem 283: 29109-29118, 2008.
48. Gupta S, Young D, Maitra RK, Gupta A, Popovic ZB, Yong SL, Mahajan A, Wang Q and Sen S: Prevention of cardiac hypertrophy and heart failure by silencing of NF-kappaB. J Mol Biol 375: 637-649, 2008.
49. Tan BK, Adya R and Randeva HS: Omentin: A novel link between inflammation, diabesity, and cardiovascular disease. Trends Cardiovasc Med 20: 143-148, 2010.
50. Rao SS, Hu Y, Xie PL, Cao J, Wang ZX, Liu JH, Yin H, Huang J, Tan YJ, Luo J, *et al*: Omentin-1 prevents inflammation-induced osteoporosis by downregulating the pro-inflammatory cytokines. Bone Res 6: 9, 2018.
51. Yoo HJ, Hwang SY, Hong HC, Choi HY, Yang SJ, Seo JA, Kim SG, Kim NH, Choi KM, Choi DS and Baik SH: Association of circulating omentin-1 level with arterial stiffness and carotid plaque in type 2 diabetes. Cardiovasc Diabetol 10: 103, 2011.
52. Genre F, Rueda-Gotor J, Remuzgo-Martínez S, Pulito-Cueto V, Corrales A, Mijares V, Lera-Gómez L, Portilla V, Expósito R, Mata C, *et al*: Omentin: A biomarker of cardiovascular risk in individuals with axial spondyloarthritis. Sci Rep 10: 9636, 2020.
53. Zhou JP, Tong XY, Zhu LP, Luo JM, Luo Y, Bai YP, Li CC and Zhang GG: Plasma omentin-1 level as a predictor of good coronary collateral circulation. J Atheroscler Thromb 24: 940-948, 2017.