Biochemical and Biophysical Research Communications 455 (2014) 126–132

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Leptin confers protection against TNF-α-induced apoptosis in rat cardiomyocytes

Lu Yu, Yanbo Zhao, Shengjie Xu, Chongying Jin, Min Wang, Guosheng Fu *

Department of Cardiovascular Medicine, Sir Run Run Shaw Hospital, College of Medicine, Zhejiang University, 3 East Qingchun Road, Hangzhou, Zhejiang 310016, China

ARTICLE INFO

Article history:
Received 23 October 2014
Available online 1 November 2014

Keywords:
Leptin
TNF-α
Apoptosis
Cardiomyocytes

ABSTRACT

Leptin, an important adipose-derived hormone, is recognized as a crucial protein in energy homeostasis. Recent studies indicated that leptin is associated with cardiac pathophysiology, however, the role and mechanisms of leptin in cardiomyocytes apoptosis are poorly understood. Here we investigated whether leptin exerted protective effect on cardiomyocytes exposed to tumor necrosis factor-alpha (TNF-α) and the possible mechanisms. Neonatal rat cardiomyocytes were subjected to TNF-α in the presence or absence of leptin. By FITC/Annexin V flow cytometry and Western blot, we noticed that TNF-α increased Annexin V binding and cleaved caspase-3/PARP, which were attenuated by leptin pretreatment. Moreover, leptin protected cardiomyocytes against mitochondrial apoptosis by inhibiting cytochrome C elevation and Bcl-2 decreasing. TNF-α-induced P38 MAPK and NF-κB activation were abolished by leptin addition, and the P38 and NF-κB inhibitor, SB203580 and Bay117082, also mitigated the apoptotic effect of TNF-α, indicating that their activation might be responsible for the apoptosis in TNF-α-treated cardiomyocytes. Therefore, leptin conferred anti-apoptotic effect in cardiomyocytes exposed to TNF-α possibly by inhibiting TNF-α-activated P38 MAPK and NF-κB pathways.

1. Introduction

Cardiomyocytes apoptosis contributes to various heart diseases as cardiomyopathy and cardiac dysfunction, which is regarded as a marker of poor cardiovascular outcomes [1–2]. The loss of myocytes increases remodeling and reduces contractile function, finally leading to heart failure. Two apoptotic pathways may be involved, including the death receptor-mediated and the intrinsic mitochondria apoptotic pathways, which are triggered by specific cell signaling molecules. It has been reported that the hormones and cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 were associated with cell apoptosis [3–4]. Elevated plasma levels or over expression of TNF-α in transgenic mice decreased Bcl-2 expression and activated the intrinsic apoptotic pathway by TNFR1 stimulation, triggering subsequent caspase cascade, eventually contributing to adverse cardiac remodeling in the adult mammalian heart [4–6].

Recently, increasing interest focused on the relationship of cardiomyopathy and obesity, which is associated with various cardiac complications such as type 2 diabetes and heart failure. However, obesity exerted a beneficial role in limiting the infarct size and cardiac remodeling after infarction [7], indicating the possible protective effect of cardiomyocytes impairment in obesity. But till date, little information is available about the effect and mechanisms of obesity and cardiac apoptosis under specific stimuli like TNF-α.

Leptin, a product of the obese gene derived from adipose tissue, has attracted much attention in recent years as a vital link between obesity and energy homeostasis [8]. The circulating levels of leptin are positively correlated with individual body mass, and it is elevated in patients with heart failure and ischemic heart disease, indicating the various biological effects of leptin in cell survival and cardiac pathophysiology regulation [9–10]. It has been demonstrated that leptin promoted cardiomyocytes hypertrophy in vitro [11], while the elevated leptin in obese mice enhanced lepR (leptin receptor) and STAT3 phosphorylation which mediated cardioprotection in angiogenesis and apoptosis [12]. As leptin deficiency or disrupted signaling might trigger cardiac dysfunction or morphologic abnormalities [13], the effect and mechanism of leptin on cardiomyocytes raised great interest in cardiovascular disease of obesity. It showed its protection against myocardial ischemia/reperfusion injury via JAK/STAT signaling activation, and by preventing caspase-3 cleavage and Bax protein translocation, leptin abrogated the H_2O_2-induced cardiomyocytes apoptosis [14–15]. But direct evidence is still absent about the effect of leptin on cardiomyocytes under inflammatory cytokines like TNF-α, and the possibly involved cell signaling mechanisms.

* Corresponding author. Fax: +86 571 86006248.
E-mail address: fuguoshengvip@sina.com (G. Fu).

http://dx.doi.org/10.1016/j.bbrc.2014.10.134
0006-291X/

In the present study, we aimed to investigate the apoptosis and cell signaling mechanisms in rat cardiomyocytes exposed to TNF-α, and whether it could be attenuated by leptin addition. Further mechanistic insight was provided via analyzing the involvement of MAPK and NF-κB pathways possibly targeted by leptin.

2. Materials and methods

2.1. Chemicals and reagents

TNF-α and Leptin were purchased from Biovision (San Francisco, USA). FITC/Annexin V Apoptosis Detection Kit (BD Bioscience, USA) for flow cytometry was used to analyze cell apoptosis. Monoclonal rabbit antibodies, such as cleaved caspase-3, caspase-3, cleaved PARP, PARP, Bcl-2 and cytochrome C were obtained from Cell Signaling Technology (Boston, MA, USA). Other antibodies, including anti-Erk, anti-phospho-Erk, anti-JNK, anti-phospho-JNK, anti-p38, anti-phospho-p38, anti-phospho-NF-κB (P65) and anti-NF-κB were also from Cell Signaling Technology. The corresponding selective inhibitors, including PD98059, SP600125, SB203580 and Bay117082 were obtained from Merck (Darmstadt, Germany). HRP-marked anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was supplied by Kangchen (Shanghai, China). Polyclonal rabbit anti-Histone-H3 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell culture

Neonatal rat cardiomyocytes were cultured, as we previously described [16]. Briefly, the heart tissue isolated from the heart of 1–2 day old Sprague–Dawley rats (provided by the experimental animal center of Zhejiang University) were dissected and digested in a Ca^{2+} and Mg^{2+} free PBS containing 0.1% trypsin and 0.1% type II collagenase for 10 min at 37 °C. DMEM cell culture medium (glucose concentration: 5.5 mM) containing 10% fetal bovine serum (FBS) was added into the collected cell supernatant to stop the digestive effect of trypsin and collagenase. The above steps were repeated until the tissues were completely digested. The cell suspension was then centrifuged and suspended in DMEM cell culture medium with 10% FBS in a humidified 5% CO_2/95% air atmosphere at 37 °C. The fibroblast in the cell suspension was reduced by pre-plating for 1 h due to the differential cell adhesion and the addition of 5′-BrdU (0.01 mM) during the first three days to inhibit the growth of fibroblast.

2.3. Protein extraction

To extract total proteins, cells in 6-well plates were harvested and washed with PBS for three times, followed by lysing on ice in 100 μL RIPA solution containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, 5% deoxycholic acid, 0.1% SDS, 1 mM EDTA, 10 mM NaF, 1 mM Na_3VO_4, 1 mM dithiothreitol, 1 mM PMSF, 2 μg/mL leupeptin for 30 min. Nuclear and cytoplasmic protein were extracted with the extraction kit supplied by Beyotime Institute of Biotechnology (Shanghai, China). Proteins were quantified with the Bio-Rad DC Protein Assay Kit II (Bio-Rad, Hercules, CA, USA).

2.4. Western blot

Samples containing equal amounts of protein were run on a 10% tris–glycine gradient gel, transferred to PVDF membranes and blocked for 1 h with 5% nonfat milk in TBST (Tris-buffered solution containing 0.1% Tween 20) at room temperature. Membranes were then soaked with primary antibodies overnight at 4 °C followed by secondary antibody incubation for 1 h at room temperature. Finally, the membranes were reacted with enhanced chemiluminescence (ECL) reagents and exposed by Image Quant LAS-4000 (Fujifilm, Tokyo, Japan). Band densities were determined by an image Multi-Gauge Software (Fujifilm, Tokyo, Japan). Each experiment was repeated at least 3 times.

2.5. Flow cytometry measurement

Cell apoptosis was quantified by a flow cytometer to determine whether leptin exerted a survival effect on cardiomyocytes. The cells were digested and harvested. FITC/Annexin V Apoptosis Detection Kit for flow cytometry was used to detect the proportion of apoptotic cells. We sought to determine the differences in early apoptotic cells, which only bind to Annexin V.

2.6. Statistical analysis

Results were expressed as mean ± SEM. for at least three individual experiments. Statistical analysis between groups was performed by one-way ANOVA. The level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. TNF-α induced upregulation of cleaved caspase-3 and cleaved PARP, which was attenuated by leptin pretreatment

Caspase-3 is one of the key executioners in cell apoptosis, and the cleavage of casepase-3 indicates its activation. It is also responsible for the proteolytic cleavage of PARP, a nuclear poly polymerase, which is involved in DNA repair and facilitates cellular disassembly. In our work, cardiomyocytes were exposed to TNF-α (10 ng/mL for 24 h) with or without the pretreatment of leptin (10 nM) for 30 min, which was a frequently used concentration in previous studies [17]. Fig. 1 showed that the TNF-α upregulated the expression of cleaved caspase-3 and cleaved PARP. Leptin pretreatment, however, attenuated the effect significantly ($P < 0.05$), indicating the protective role of leptin in TNF-α-induced cardiomyocytes apoptosis.

3.2. Leptin protected the cardiomyocytes from TNF-α-induced mitochondria apoptosis

Mitochondria apoptosis is a critical step in the progression of intrinsic apoptotic pathway. In our study, TNF-α increased the release of cytochrome C from mitochondria in cytoplasma, which is known to bind with the adaptor apoptotic protease activator factor-I (APAFI) and recruit caspase-9/3 that ultimately lead to cell apoptosis [18]. The loss of bcl-2, a protective protein in mitochondria, was also observed in cells treated by TNF-α. With the pretreatment of leptin, however, both the effects of TNF-α could be mitigated (Fig. 2A and B). The result implicated the protective role of leptin in mitochondria apoptosis and the mechanism might involve the recovery of bcl-2 and cytochrome c. In Fig. 2C and D, the leptin alone showed no effect in the expression of cleaved caspase-3 or cytochrome C.

3.3. Leptin might prevent apoptosis via P38 MAPK and NF-κB pathways

Signal transduction in cardiomyocytes could be mediated by several important pathways, including MAPKs and NF-κB pathways, which have been reported as major signaling systems in response to TNF-α. We investigated the phosphorylation levels of

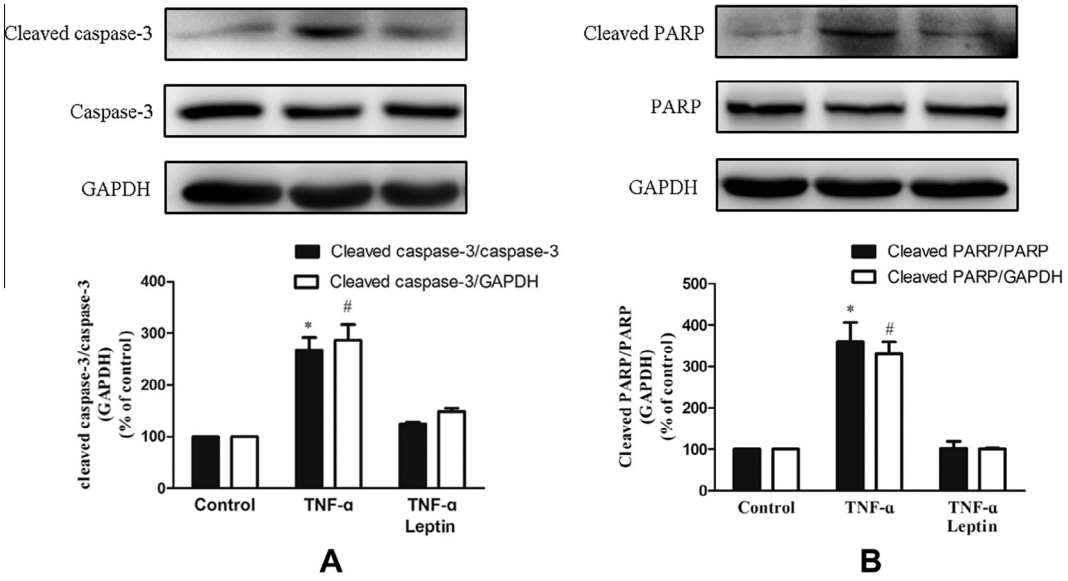

Fig. 1. Effects of TNF-α (10 ng/mL for 24 h) or leptin (10 nM) pretreatment for 30 min on caspase-3 (A) and PARP cleavage (B) in rat cardiomyocytes determined by Western blot ($n = 3$ in each experiment). $^{*}P < 0.05$ vs. control (normalized by total caspase-3 or PARP). $^{\#}P < 0.05$ vs. control (normalized by GAPDH). Data are mean ± SEM.

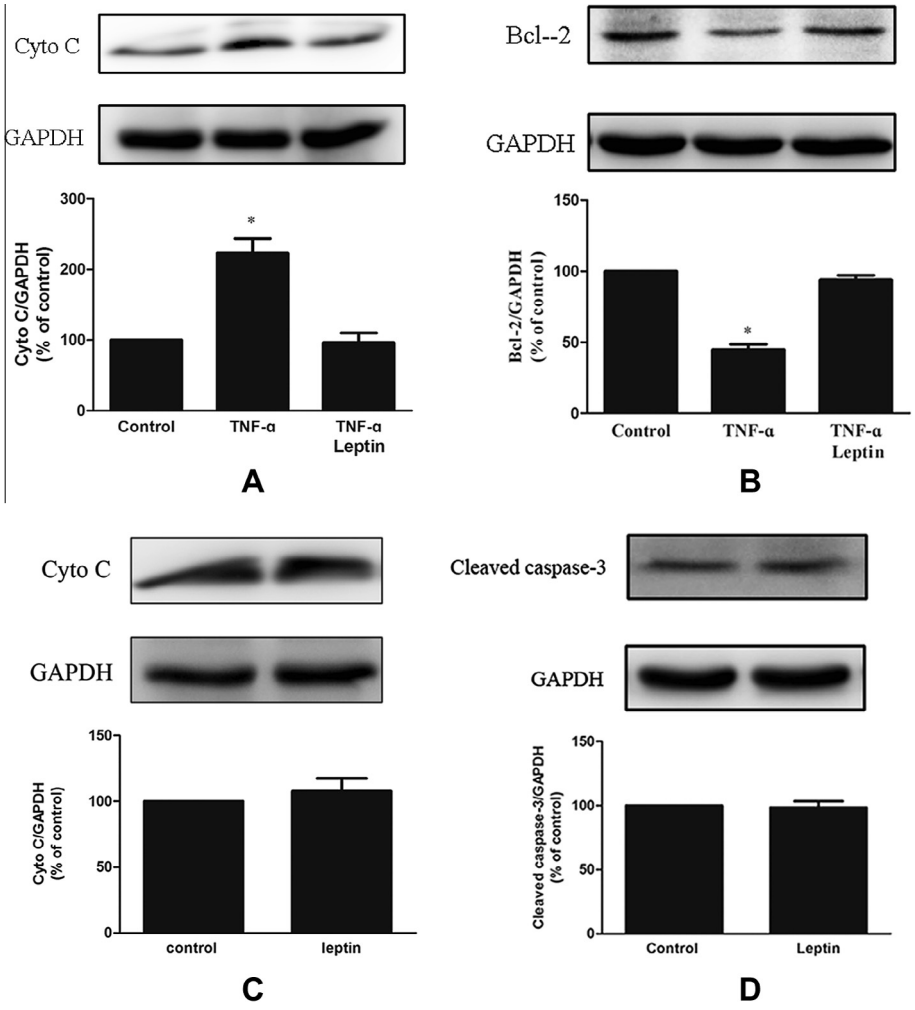

Fig. 2. Western blot analysis of the effect of TNF-α (10 ng/mL for 24 h) or leptin (10 nM) pretreatment for 30 min on cytochrome C (Cyto C) release in cytoplasma (A) and Bcl-2 expression (B). C and D showed the analysis of the expression of Cyto C and Bcl-2 protein expression by leptin addition alone. $P < 0.05$ vs. control. Data are mean ± SEM ($n = 3$ in each experiment).

MAPK family proteins (Erk, P38, JNK) and nuclear NF-κB (P65) in cardiomyocytes treated by TNF-α for 30 min with or without the presence of leptin pre-incubation. Their expressions were measured by Western blot (Fig. 3A–E). It showed that the phosphorylation levels of Erk, JNK, P38 MAPK and nuclear NF-κB were significantly increased by TNF-α after 30 min, but only phospho-P38 and phospho-NF-κB could be recovered by leptin pre-treatment. We then added SB203580, the specific P38 inhibitor 30 min before TNF-α, and it was observed that the inhibited phospho-P38 level was followed by reduced nuclear phospho-NF-κB, indicating that phosphorylation levels of nuclear NF-κB might be partly regulated by P38 MAPK pathway.

As TNF-α activated both MAPK and NF-κB pathways, we tried to investigated the specific signal transduction pathways involved in the anti-apoptotic role of leptin. Cardiomyocytes incubated by TNF-α (10 ng/mL for 24 h) were then pre-treated with leptin (10 nM), or NF-κB inhibitor Bay117082 (10 μM), p38 MAPK inhibitor SB203580 (10 μM), or Erk MAPK inhibitor PD98059 (20 μM), or JNK MAPK inhibitor SP600125 (20 μM). All the inhibitors were added to cells 30 min before TNF-α treatment. By Western blot (Fig. 3F), it was observed that the TNF-α-induced cleaved caspase-3 elevation was nearly abolished by leptin or Bay117082 or SB203580 pre-treatment, but not by PD98059 or SP600125. In Fig. 4, the similar protective effect of Bay117082 and leptin on TNF-α-induced apoptosis was exhibited by flow cytometry with FITC/Annexin V Apoptosis Detection Kit.

According to the results in Figs. 3 and 4 indicating that leptin attenuated the expression of activated P38 and nuclear NF-κB, we proposed that leptin might inhibit the TNF-α-induced apoptosis partly by blocking the activation of P38 MAPK and NF-κB pathways.

4. Discussion

Excess TNF-α levels have been documented to cause myocyte apoptosis when binding to cell TNFR1, leading to the loss of cardiomyocytes and various cardiovascular diseases [4,6,19]. Activated nuclear NF-κB and mitochondria impairment facilitate the inflammatory response and act as key regulators in the cell signaling

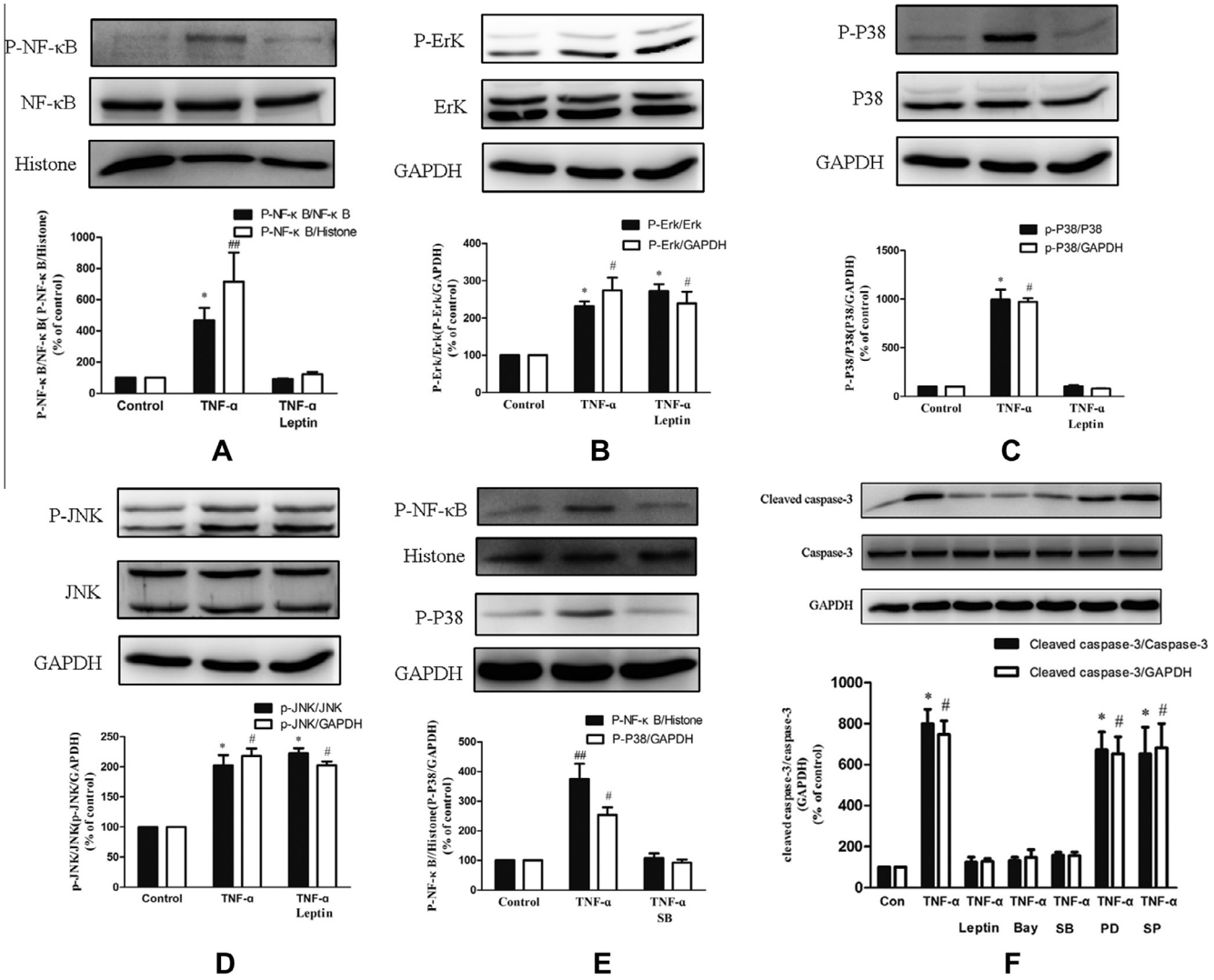

Fig. 3. The cell signaling transduction of TNF-α or leptin pretreatment in rat cardiomyocytes analyzed by Western blot. (A–D) Showed the phosphorylation levels of nuclear NF-κB, Erk, P38 and JNK MAPK exposed by TNF-α (10 ng/mL) for 30 min with or without the presence of leptin (10 nM) pretreatment for 30 min. The results were normalized by total nuclear NF-κB or MAPK. We also used Histone as an internal control to normalize the nuclear protein expression, and GAPDH for plasma protein. (E) The specific P38 inhibitor, SB203580, was added 30 min before TNF-α incubation (10 ng/mL, 30 min) in order to detect the expression of phosphor-P38 and nuclear phosphor-NF-κB, which were normalized by GAPDH and Histone, respectively. (F) Western blot analysis of the caspase-3 cleavage in TNF-α-treated (10 ng/mL for 24 h) cardiomyocytes with the pre-incubation of leptin (10 nM) or NF-κB inhibitor or specific MAPK inhibitors for 30 min, respectively. $^{*}P < 0.05$ vs. control (normalized by total MAPK or caspase-3). $^{\#}P < 0.05$ vs. control (normalized by GAPDH). $^{\#\#}P < 0.05$ vs. control (normalized by Histone). Data are mean ± SEM ($n = 3$ in each experiment).

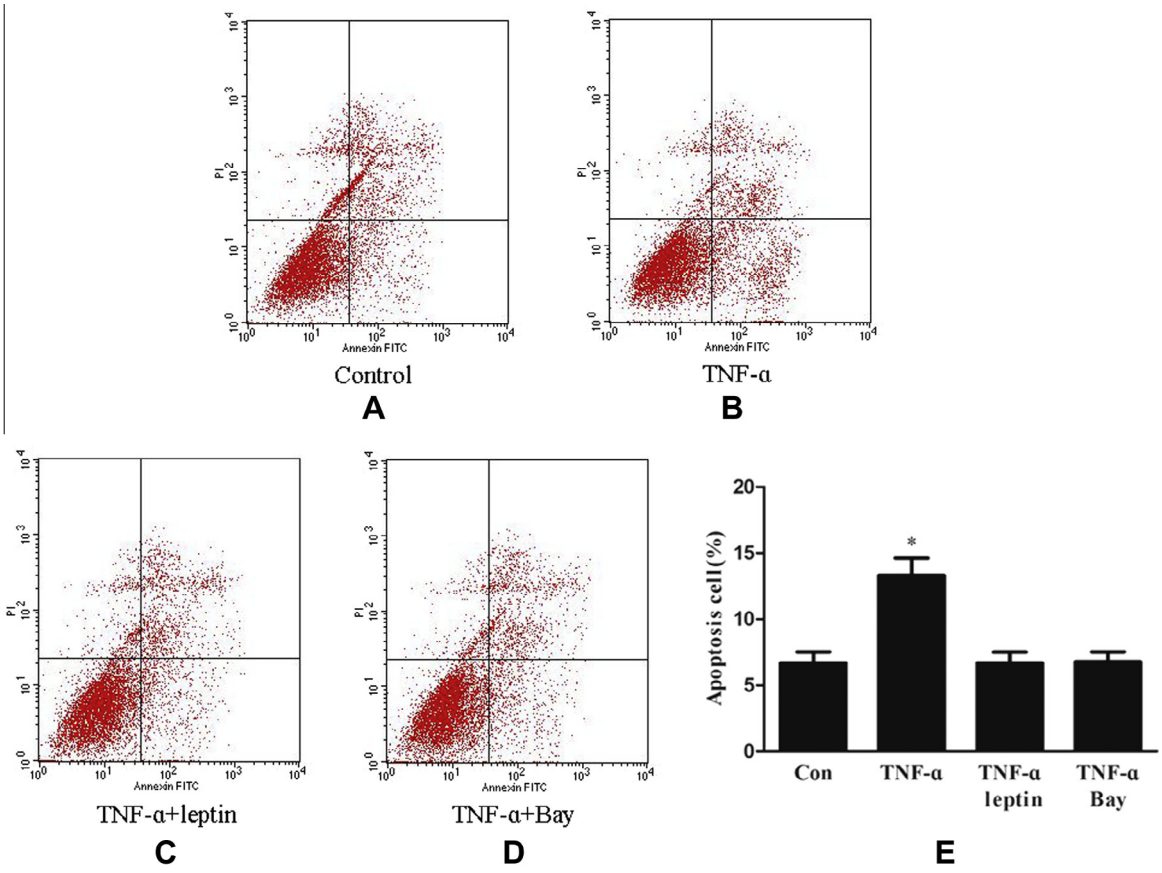

Fig. 4. Flow cytometry after staining with FITC/Annexin V and propodium iodide were performed to determine the apoptosis of cardiomyocytes, which were exposed to TNF-α (10 ng/mL, 24 h) or with leptin (10 nM) or NF-κB inhibitor (Bay117082, 10 μM) pretreatment for 30 min, as shown in A–D. (E) Showed the quantity analysis of the cell apoptotic rate in each group. $^{*}P < 0.05$ vs. control. Data are mean ± SEM ($n = 3$ in each experiment).

mechanism [20]. In our study, we investigated the effect and mechanism of leptin, a product of the obese (ob) gene, on TNF-α-induced apoptosis in neonatal rat cardiomyocytes, which was still poorly available to understand the precise targets of leptin in regulating the pathophysiology under inflammatory conditions in obesity.

Our data showed that leptin attenuated the TNF-α-induced myocyte apoptosis by blocking the activation of the caspase-3 cleavage and intrinsic mitochondria pathways. It also abolished the phosphorylation of P38 MAPK/NF-κB upregulated by TNF-α, which might be responsible for TNF-α-induced apoptosis.

TNF-α is a pro-inflammatory cytokine that is involved in the pathogenic conditions such as cardiovascular injury and disease states. Studies have demonstrated that elevated plasma levels or over expression of TNF-α could provoke the cardiomyocytes oxidative stress and apoptosis by TNFR1 stimulation with subsequent caspase cascade triggering [5,21]. We used neonatal rat cardiomyocytes as the experimental model. Exposure to TNF-α increased the activation of caspase-3 cleavage. The cleaved caspase-3 is a 17-kDa proteolytic fragment activated from caspase-3 and serves as an early marker of cell apoptosis. We further detected its substrate, PARP, and noticed that the proteolysis of PARP was increased by TNF-α as illustrated in Fig. 1B. It showed that TNF-α could trigger caspase cascade in neonatal rat cardiomyocytes, which might lead to myocyte apoptosis that was approved by flow cytometry measurement in our study.

As the mitochondria impairment also participates in the apoptosis pathway, we extracted plasma proteins from cardiomyocytes, and observed that TNF-α induced a significant cytochrome C release to cytosol. It is a critical step in apoptotic cell death that is associated with caspase-3 activation. Bcl-2, an anti-apoptotic protein, however, was inhibited by TNF-α. The results implicated that TNF-α might also take its effect via intrinsic apoptotic pathway. We then pre-incubated the cells with leptin, an obese gene product elevated in obesity, to observe whether it could ameliorate the TNF-α-induced myocyte apoptosis. Recent studies has pointed out that leptin was involved in cardioprotection besides its effect on energy and metabolic balance [22–23]. It reduced the infarct size and protected the heart against ischemia/reperfusion injury [17]. And in vitro models [14], its protection against myocardial ischemia/reperfusion injury involved JAK/STAT signaling pathway activation and inhibition of mitochondrial permeability transition pore (MPTP) opening. In H9C2 cells [15], leptin pretreatment showed its anti-apoptotic role by preventing H_2O_2-induced increases in caspase-3 cleavage and Bax protein translocation. By enhancing SOD activity, a major anti-oxidant enzyme, leptin could also inhibit the serum-deprivation-induced apoptosis in cardiomyocytes [24]. But little information was available to investigate the effect of leptin on TNF-α-induced myocyte apoptosis and the involved mechanisms. In our study, leptin prevented both the TNF-α-induced fragmentation of caspase-3 and PARP. Increased cytochrome C release was reduced, while the inhibited Bcl-2 expression was restored. The flow cytometry also approved that the apoptosis induced by TNF-α was ameliorated by leptin pretreatment. It showed that leptin might attenuate the TNF-α-induced myocyte apoptosis possibly by blocking caspase-3 activation and intrinsic apoptotic pathway. In addition, leptin alone did not exert any effect on caspase-3 cleavage or cytochrome C release, indicating the mechanisms should still be further investigated.

A recent study [25] mentioned that exposure to TNF-α significantly increased myocyte ROS production and cell injury, and the

change was associated with an increase in the P38 phosphorylation and a decrease in the phospho-ERK1/2 levels. Pre-incubation with P38 inhibitor SB203580 abrogated the TNF-α-induced changes. Chen et al. [26] suggested that H_2O_2-induced secretion of TNF-α also increased cardiomyocyte apoptosis through ROS-dependent activation of P38 MAPK. It was comparable with our results that the upregulated phospho-P38 might contribute to TNF-α-induced myocyte injury. In our work, TNF-α treatment for 30 min also activated the expression of phospho-ERK1/2, phospho-JNK and nuclear phospho-NF-κB. The result of the activated phospho-ERK1/2 was different with the previous study [25] demonstrating a decrease in the phospho-ERK1/2 by TNF-α, in which the authors used ventricular myocytes isolated from adult male SD rats as experimental models and detected the MAPK phosphorylation levels 4 h after TNF-α treatment. We added P38 inhibitor SB203580 before TNF-α exposure, and noticed that SB203580 inhibited not only the phospho-P38 levels, but also the activation of nuclear phospho-NF-κB, indicating that nuclear NF-κB might be in the downstream cascade and regulated by P38 MAPK pathway.

Leptin pretreatment before TNF-α, however, abolished the activation of phospho-P38 and nuclear phospho–NF-κB. While the TNF-α-induced phospho-ERK1/2 and phospho-JNK expression could not be recovered by leptin. To further investigate the protective mechanism of leptin, we added the MAPK and NF-κB inhibitors separately before TNF-α incubation. According to Fig. 3F, only P38 and NF-κB inhibitors (SB203580 and Bay117082) abrogated the effect of TNF-α as leptin did, implicating that the blockage of P38 MAPK/NF-κB activation might be partly involved in the protective mechanisms of leptin in TNF-α-induced myocyte apoptosis. NF-κB is a transcription factor that participates in cell survival, growth and inflammation [27]. The activation and translocation of p65 NF-κB to nucleus could bind with DNA to regulate gene expression, which is responsible for facilitating ischemia/reperfusion injury and ROS-dependent cell apoptosis [28–31]. It was consistent with our results that mainly detected the nuclear phosphorylation levels of p65 NF-κB. But some other evidences suggested conflicting results that NF-κB activation might have cardioprotection under ischemia [32] as NF-κB is also a vital factor maintaining our innate immunity. The NF-κB has cardioprotection in the late phase after ischemic preconditioning, but contributes to myocyte injury after ischemia/reperfusion [33–34]. It has been reported that there might be a NF-κB-dependent gene network initiated after specific stimuli, and the effect of the intermediate and late primary target genes might differ [32,35]. In our work, as shown in Figs. 3 and 4, the blockage of NF-κB activation by SB203580 or Bay117082 or leptin abrogated the TNF-α-induced myocyte apoptosis, indicating that the TNF-α-induced NF-κB activation might be injurious in cardiomyocytes.

Several previous studies mentioned of the protective mechanism of leptin and cell signaling pathways in cardiomyocytes. It has been demonstrated that JAK/STAT signaling [14] was involved in the anti-apoptotic effect of leptin in myocardial ischemia/reperfusion injury, and increased phosphorylation of P38 MAPK and AMPK also contributed to the protective mechanism of leptin in ischemia/reperfusion models [36]. LPS-induced P38 MAPK activation has been reported to be attenuated by leptin pretreatment which eventually mitigated the cell apoptosis [37]. Similar effects of leptin could also been seen in high-glucose-induced P38 phosphorylation and cell injury [38]. According to our results, leptin pretreatment abolished TNF-α-induced P38 MAPK/NF-κB activation, which contributed to the apoptosis process in cardiomyocytes. Therefore, the data presented here suggested that the activated P38 MAPK/NF-κB might be vital targets for leptin protecting against TNF-α-induced myocytes injury. Further studies should focus on the target downstream gene expressions of NF-κB in TNF-α-treated cardiomyocytes and the related mechanisms of intrinsic mitochondria apoptotic pathways.

5. Conclusion

Our study provided the first evidence that the obesity-associated hormone, leptin, abrogated the TNF-α-induced apoptosis in neonatal rat cardiomyocytes possibly by blocking the activation of the caspase-3 cleavage and intrinsic mitochondria pathways. Leptin also abolished the phosphorylation of P38 MAPK/NF-κB upregulated by TNF-α, which might be involved in TNF-α-induced apoptosis.

Acknowledgments

This study was supported by Natural Science Funds of Zhejiang province, China (No. LY13H020002).

References

[1] N. Guttenplan, C. Lee, W.H. Frishman, Inhibition of myocardial apoptosis as a therapeutic target in cardiovascular disease prevention: focus on caspase inhibition, Heart Dis. 3 (2001) 313–318.
[2] P.M. Kang, S. Izumo, Apoptosis and heart failure: a critical review of the literature, Circ. Res. 86 (2000) 1107–1113.
[3] K.T. Moe, K. Khairunnisa, N.O. Yin, J. Chin-Dusting, P. Wong, M.C. Wong, Tumor necrosis factor-alpha-induced nuclear factor-kappaB activation in human cardiomyocytes is mediated by NADPH oxidase, J. Physiol. Biochem. 70 (2014) 769–779.
[4] D. Engel, R. Peshock, R.C. Armstong, N. Sivasubramanian, D.L. Mann, Cardiac myocyte apoptosis provokes adverse cardiac remodeling in transgenic mice with targeted TNF overexpression, Am. J. Physiol. Heart Circ. Physiol. 287 (2004) H1303–H1311.
[5] M. Sugano, T. Hata, K. Tsuchida, N. Suematsu, J. Oyama, S. Satoh, N. Makino, Local delivery of soluble TNF-alpha receptor 1 gene reduces infarct size following ischemia/reperfusion injury in rats, Mol. Cell. Biochem. 266 (2004) 127–132.
[6] M. Packer, Is tumor necrosis factor an important neurohormonal mechanism in chronic heart failure?, Circulation 92 (1995) 1379–1382
[7] A. Habbu, N.M. Lakkis, H. Dokainish, The obesity paradox: fact or fiction?, Am J. Cardiol. 98 (2006) 944–948.
[8] M. Balasko, S. Soos, M. Szekely, E. Petervari, Leptin and aging: review and questions with particular emphasis on its role in the central regulation of energy balance, J. Chem. Neuroanat. (2014) (Epub ahead of print).
[9] W. Doehner, M. Rauchhaus, I.F. Godsland, K. Egerer, J. Niebauer, R. Sharma, M. Cicoira, V.G. Florea, A.J. Coats, S.D. Anker, Insulin resistance in moderate chronic heart failure is related to hyperleptinaemia, but not to norepinephrine or TNF-alpha, Int. J. Cardiol. 83 (2002) 73–81.
[10] P.C. Schulze, J. Kratzsch, A. Linke, N. Schoene, V. Adams, S. Gielen, S. Erbs, S. Moebius-Winkler, G. Schuler, Elevated serum levels of leptin and soluble leptin receptor in patients with advanced chronic heart failure, Eur. J. Heart Fail. 5 (2003) 33–40.
[11] F.P. Xu, M.S. Chen, Y.Z. Wang, Q. Yi, S.B. Lin, A.F. Chen, J.D. Luo, Leptin induces hypertrophy via endothelin-1-reactive oxygen species pathway in cultured neonatal rat cardiomyocytes, Circulation 110 (2004) 1269–1275.
[12] M. Leifheit-Nestler, N.M. Wagner, R. Gogiraju, M. Didie, S. Konstantinides, G. Hasenfuss, K. Schafer, Importance of leptin signaling and signal transducer and activator of transcription-3 activation in mediating the cardiac hypertrophy associated with obesity, J. Transl. Med. 11 (2013) 170.
[13] J. Ren, H. Ma, Impaired cardiac function in leptin-deficient mice, Curr. Hypertens. Rep. 10 (2008) 448–453.
[14] C.C. Smith, R.A. Dixon, A.M. Wynne, L. Theodorou, S.G. Ong, S. Subrayan, S.M. Davidson, D.J. Hausenloy, D.M. Yellon, Leptin-induced cardioprotection involves JAK/STAT signaling that may be linked to the mitochondrial permeability transition pore, Am. J. Physiol. Heart Circ. Physiol. 299 (2010) H1265–H1270.
[15] M. Eguchi, Y. Liu, E.J. Shin, G. Sweeney, Leptin protects H9c2 rat cardiomyocytes from H_2O_2-induced apoptosis, FEBS J. 275 (2008) 3136–3144.
[16] L. Yu, Y. Zhao, Y. Fan, M. Wang, S. Xu, G. Fu, Epigallocatechin-3 gallate, a green tea catechin, attenuated the downregulation of the cardiac gap junction induced by high glucose in neonatal rat cardiomyocytes, Cell. Physiol. Biochem. 26 (2010) 403–412.
[17] C.C. Smith, M.M. Mocanu, S.M. Davidson, A.M. Wynne, J.C. Simpkin, D.M. Yellon, Leptin, the obesity-associated hormone, exhibits direct cardioprotective effects, Br. J. Pharmacol. 149 (2006) 5–13.
[18] N.N. Danial, S.J. Korsmeyer, Cell death: critical control points, Cell 116 (2004) 205–219.
[19] O. Micheau, J. Tschopp, Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes, Cell 114 (2003) 181–190.

[20] S. Dhingra, A.K. Sharma, R.C. Arora, J. Slezak, P.K. Singal, IL-10 attenuates TNF-alpha-induced NF kappaB pathway activation and cardiomyocyte apoptosis, Cardiovasc. Res. 82 (2009) 59–66.
[21] R. Aikawa, Y. Nitta-Komatsubara, S. Kudoh, H. Takano, T. Nagai, Y. Yazaki, R. Nagai, I. Komuro, Reactive oxygen species induce cardiomyocyte apoptosis partly through TNF-alpha, Cytokine 18 (2002) 179–183.
[22] D.J. Hausenloy, D.M. Yellon, New directions for protecting the heart against ischaemia–reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway, Cardiovasc. Res. 61 (2004) 448–460.
[23] J. Ren, Leptin and hyperleptinemia – from friend to foe for cardiovascular function, J. Endocrinol. 181 (2004) 1–10.
[24] J. Zheng, J. Fang, Y.J. Yin, X.C. Wang, A.J. Ren, J. Bai, X.J. Sun, W.J. Yuan, L. Lin, Leptin protects cardiomyocytes from serum-deprivation-induced apoptosis by increasing anti-oxidant defence, Clin. Exp. Pharmacol. Physiol. 37 (2010) 955–962.
[25] S. Dhingra, A.K. Sharma, D.K. Singla, P.K. Singal, P38 and ERK1/2 MAPKs mediate the interplay of TNF-alpha and IL-10 in regulating oxidative stress and cardiac myocyte apoptosis, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H3524–H3531.
[26] Z. Chen, H. Jiang, Y. Wan, C. Bi, Y. Yuan, H(2)O(2)-induced secretion of tumor necrosis factor-alpha evokes apoptosis of cardiac myocytes through reactive oxygen species-dependent activation of p38 MAPK, Cytotechnology 64 (2012) 65–73.
[27] A.S. Baldwin Jr., Series introduction: the transcription factor NF-kappaB and human disease, J. Clin. Invest. 107 (2001) 3–6.
[28] G. Valen, Signal transduction through nuclear factor kappa B in ischemia–reperfusion and heart failure, Basic Res. Cardiol. 99 (2004) 1–7.
[29] N.C. Moss, W.E. Stansfield, M.S. Willis, R.H. Tang, C.H. Selzman, IKKbeta inhibition attenuates myocardial injury and dysfunction following acute ischemia–reperfusion injury, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H2248–H2253.
[30] J.W. Kim, Y.C. Jin, Y.M. Kim, S. Rhie, H.J. Kim, H.G. Seo, J.H. Lee, Y.L. Ha, K.C. Chang, Daidzein administration in vivo reduces myocardial injury in a rat ischemia/reperfusion model by inhibiting NF-kappaB activation, Life Sci. 84 (2009) 227–234.
[31] S. Kumar, S. Prasad, S.L. Sitasawad, Multiple antioxidants improve cardiac complications and inhibit cardiac cell death in streptozotocin-induced diabetic rats, PLoS ONE 8 (2013) e67009.
[32] M.E. Wilhide, M. Tranter, X. Ren, J. Chen, M.A. Sartor, M. Medvedovic, W.K. Jones, Identification of a NF-kappaB cardioprotective gene program: NF-kappaB regulation of Hsp70.1 contributes to cardioprotection after permanent coronary occlusion, J. Mol. Cell. Cardiol. 51 (2011) 82–89.
[33] M. Tranter, X. Ren, T. Forde, M.E. Wilhide, J. Chen, M.A. Sartor, M. Medvedovic, W.K. Jones, NF-kappaB driven cardioprotective gene programs; Hsp70.3 and cardioprotection after late ischemic preconditioning, J. Mol. Cell. Cardiol. 49 (2010) 664–672.
[34] M. Brown, M. McGuinness, T. Wright, X. Ren, Y. Wang, G.P. Boivin, H. Hahn, A.M. Feldman, W.K. Jones, Cardiac-specific blockade of NF-kappaB in cardiac pathophysiology: differences between acute and chronic stimuli in vivo, Am. J. Physiol. Heart Circ. Physiol. 289 (2005) H466–H476.
[35] M.H. Sung, L. Salvatore, R. De Lorenzi, A. Indrawan, M. Pasparakis, G.L. Hager, M.E. Bianchi, A. Agresti, Sustained oscillations of NF-kappaB produce distinct genome scanning and gene expression profiles, PLoS ONE 4 (2009) e7163.
[36] E.J. Shin, K. Schram, X.L. Zheng, G. Sweeney, Leptin attenuates hypoxia/reoxygenation-induced activation of the intrinsic pathway of apoptosis in rat H9c2 cells, J. Cell. Physiol. 221 (2009) 490–497.
[37] C. Liang, J. Liao, Z. Deng, C. Song, J. Zhang, L. Zabeau, J. Tavernier, K. Zhang, H. Xue, G. Yan, Leptin attenuates lipopolysaccharide-induced apoptosis of thymocytes partially via down-regulation of cPLA2 and p38 MAPK activation, Int. Immunopharmacol. 15 (2013) 620–627.
[38] X.D. Zhuang, X. Hu, M. Long, X.B. Dong, D.H. Liu, X.X. Liao, Exogenous hydrogen sulfide alleviates high glucose-induced cardiotoxicity via inhibition of leptin signaling in H9c2 cells, Mol. Cell. Biochem. 391 (2014) 147–155.