Amino Acids (2013) 44:961–968
DOI 10.1007/s00726-012-1425-5

ORIGINAL ARTICLE

Vaspin attenuates the apoptosis of human osteoblasts through ERK signaling pathway

Xiao Zhu · Yi Jiang · Peng-Fei Shan · Jie Shen · Qiu-Hua Liang · Rong-Rong Cui · Yuan Liu · Guan-Ying Liu · Shan-Shan Wu · Qiong Lu · Hui Xie · You-Shuo Liu · Ling-Qing Yuan · Er-Yuan Liao

Received: 6 July 2012 / Accepted: 22 October 2012 / Published online: 8 November 2012

Abstract It has been hypothesized that adipocytokines originating from adipose tissue may have an important role in bone metabolism. Vaspin is a novel adipocytokine isolated from visceral white adipose tissue, which has been reported to have anti-apoptotic effects in vascular endothelial cells. However, to the best of our knowledge there is no information regarding the effects of vaspin on osteoblast apoptosis. This study therefore examined the possible effects of vaspin on apoptosis in human osteoblasts (hOBs). Our study established that vaspin inhibits hOBs apoptosis induced by serum deprivation, as determined by ELISA and TUNEL assays. Western blot analysis revealed that vaspin upregulates the expression of Bcl-2 and downregulates that of Bax in a dose-dependent manner. Vaspin stimulated the phosphorylation of ERK, and pretreatment of hOBs with the ERK inhibitor PD98059 blocked the vaspin-induced activation of ERK, however, vaspin did not stimulate the phosphorylation of p38, JNK or Akt. Vaspin protects hOBs from serum deprivation-induced apoptosis, which may be mediated by activating the MAPK/ERK signaling pathway.

Keywords Vaspin · Osteoblast · Apoptosis · Extracellular signal-regulated kinase

Introduction

Numerous studies indicate that body fat plays an important role in bone mass (Chen et al. 2010; Cao 2011; Holecki and Wiecek 2010; Zaidi et al. 2012). Mechanical loading may affect bone mass, although other factors are also thought to contribute to this relationship. Adipose tissue is considered to be the largest endocrine organ, secreting adipocytokines such as leptin, adiponectin and apelin. Previous studies have demonstrated that plasma leptin levels are positively

X. Zhu and Y. Jiang contributed equally to this work.

X. Zhu · Q.-H. Liang · R.-R. Cui · Y. Liu · G.-Y. Liu · S.-S. Wu · H. Xie · L.-Q. Yuan (✉) · E.-Y. Liao (✉)
Institute of Metabolism and Endocrinology, The Second Xiang-Ya Hospital, Central South University, Changsha, Hunan, People's Republic of China
e-mail: allenylq@hotmail.com

E.-Y. Liao
e-mail: eyliao007@yahoo.com.cn

X. Zhu · J. Shen
Department of Metabolism and Endocrinology, The Third Affiliated Hospital, Southern Medical University, Guangzhou, Guangdong 510630, People's Republic of China

Y. Jiang
Department of Pathology, The Second Xiang-Ya Hospital, Central South University, Changsha, Hunan, People's Republic of China

P.-F. Shan
Department of Endocrinology and Metabolism, The Second Affiliated Hospital, ZheJiang University College of Medicine, Hangzhou, Zhejiang, People's Republic of China

Q. Lu
Institute of Clinical Pharmacy and Pharmacology, Second Xiang-Ya Hospital, Central South University, Changsha, Hunan, People's Republic of China

Y.-S. Liu
Geriatric Department, Second Xiang-Ya Hospital, Central South University, Changsha, Hunan, People's Republic of China

associated with bone mineral density (BMD), while serum adiponectin levels are negatively related to BMD (Biver et al. 2011). Our recent work demonstrated that apelin inhibits osteoblast apoptosis (Xie et al. 2007). However, the functions of adipocytokines in bone metabolism are presently insufficiently understood.

Vaspin (visceral adipose tissue-derived serine proteinase inhibitor), a novel adipocytokine, is derived from visceral white adipose tissues of the Otsuka Long-Evans Tokushima fatty rat, an animal model of type 2 diabetes (Hida et al. 2005). Vaspin has multiple physiological properties such as improving glucose tolerance, increasing insulin sensitivity, lowering blood glucose level, decreasing food intake and acting as an anti-inflammatory in animal or in vitro study (Wada 2008; Brunetti et al. 2011; Klöting et al. 2011; Phalitakul et al. 2011). However, clinical studies have demonstrated that increased circulating vaspin levels are associated with obesity and insulin resistance (Tan et al. 2008; Gulcelik et al. 2009). The increased serum vaspin in obesity and obese type 2 diabetes mellitus is possibly related to vaspin resistance similar to the effect of leptin (Hamed et al. 2011; Mohiti et al. 2009; Lee et al. 2011). However, there are no data regarding the potential effect of vaspin on osteoblasts. Our present study investigates the effect of vaspin on apoptosis of human osteoblasts (hOBs). Our results suggest that vaspin attenuates osteoblast apoptosis through activation of the extracellular signal-related protein kinase (ERK) pathway.

Materials and methods

Reagents

Recombinant human vaspin was purchased from American Peprotech Inc. (Rocky Hill, NJ, USA). Anti-human Bcl-2, Bax, β-actin, T-ERK1/2, p-ERK1/2, p38, p-p38, JNK, p-JNK, Akt, and p-Akt antibodies and anti-mouse and rabbit IgG peroxidase conjugated antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). The ERK inhibitor PD98059 was purchased from Calbiochem Corp. (San Diego, CA, USA).

Cell cultures

Osteoblasts were obtained from traffic accident victims undergoing surgery and this procedure was approved by the local research ethics committee as previously described (Liu et al. 2010). hOBs isolated from femur were cultured in α-MEM medium (Gibico-BRL. Corp., NY, USA) containing 15 % fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5 % CO_2 atmosphere. Cells were confirmed as osteoblasts by the expression of ALP, collagen type I and osteocalcin, and formation of mineralized nodules as our previously described (Xie et al. 2006), when cultured in media containing 50 μg/ml acrobatic acids, 200 nM dexamethasone and 10 mM β-glycerophosphate at 37 °C in a 5 % CO_2 atmosphere. To assay the effect of ERK inhibition on hOB apoptosis, hOBs were pretreated with 10 μM ERK inhibitor PD98059 for 3 h prior to treatment with 100 ng/ml vaspin for 48 h.

Cell apoptosis measurement

Cell death ELISA measurement

Apoptosis was directly detected by measuring cytoplasmic nucleosomes (i.e. DNA complexed with histone in the cytoplasm) with a Cell Death Detection ELISA kit (Roche Diagnostics, Roche Molecular Biochemicals, Mannheim, Germany) as described in the manufacturer's instructions. Osteoblasts were seeded at a density of 1.0×10^4 cells/well in 24-well plates for 24 h. After culturing in serum-free medium for 24 h, hOBs were exposed to various concentrations (0, 1, 10, 100 ng/ml) of vaspin for 48 h in the absence of serum. Cells were then washed by PBS and incubated for 30 min with 0.5 ml of lysis buffer at 4 °C and then centrifuged at 15,000 rpm for 10 min. Nucleosomes detected in the supernatants indicated the extent of apoptosis in the sample. To assay the effect of kinase inhibitor on hOB apoptosis, hOBs were pretreated with 10 μM ERK inhibitor PD98059 for 3 h prior to treatment with 100 ng/ml vaspin for 48 h.

TUNEL staining detection of hOBs apoptosis

In situ apoptosis of hOBs was measured by terminal deoxynucleotidyl transferase-mediated deoxyribonucleotide triphosphate nick end-labeling (TUNEL). Cells were cultured in serum-free medium for 24 h, and then treated with absence or presence of 100 ng/ml vaspin for 48 h. The cells were washed three times with PBS, and then stained with TUNEL reagent (Roche Diagnostics), according to the manufacturer's protocol. DAPI (4-diamino-2-phenylindole) was used to counterstain the nuclei. Cells were observed using a fluorescence microscope (×400 magnification). Six fields were randomly selected and the percentage of positive cells was calculated as the apoptosis index (AI) using the following equation: AI = (number of positive cells/total number of cells) × 100 %, as previously described (Kitamura et al. 2004).

Western blot analysis

Osteoblasts were seeded for 24 h followed by culturing for 24 h in serum-free medium. The cells were then treated

with or without 100 ng/ml vaspin for 48 h. Immunoblotting was performed as previously described (Yuan et al. 2007, 2010). In brief, total protein was extracted using RIPA lysis buffer (Beyotime, China). A Bradford assay was used to determine protein concentration, and equal amounts of protein were loaded onto SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5 % non-fat milk in PBS for 1 h, and then incubated with anti-Bcl-2 antibody, anti-Bax antibody, or anti-β-actin antibodies. Resultant protein bands following incubation with an appropriate secondary antibody were visualized by chemiluminescence.

Measurement of MAPK and PI3-K/Akt activation

hOBs were exposed to 100 ng/ml vaspin for 0–60 min. Cell layers were rinsed twice with cold PBS containing 5 mM EDTA and 0.1 mM Na_3VO_4, and lysed with a buffer consisting of 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 % Triton X-100, 10 mM NaH_2PO_4, 10 % glycerol, 2 mM Na_3VO_4, 10 mM NaF, 1 mM ABSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. Western blot analysis was performed as before (Yuan et al. 2007, 2010). Equal amount of protein was transferred onto PVDF membranes. The membranes were incubated with anti-p-ERK1/2, T-ERK1/2, p-p38, p-38, p-JNK, JNK, p-Akt and Akt antibodies and visualized by chemiluminescence.

Statistical analysis

Data are presented as the mean ± standard deviation (SD). Differences between groups were compared by one-way ANOVA. The results shown are based on at least three independent experiments.

Results

Vaspin inhibits hOBs apoptosis induced by serum deprivation

Serum-deprived hOBs were treated with vaspin to determine its effect on apoptosis. Cell Death ELISA indicated that after treatment with various concentrations of vaspin (1, 10, 100 ng/ml), the incidence of hOBs apoptosis induced by serum deprivation was significantly lower than that of control cells (1 ng/ml: 2.88 ± 0.18; 10 ng/ml: 2.40 ± 0.21; 100 ng/ml: 1.90 ± 0.15; and control cell: 3.32 ± 0.19 ELISA absorbance units respectively; $p < 0.05$). We note that there was a consistent decrease in apoptosis with increasing concentrations of vaspin, up to 100 ng/ml. In this regard, suppression of hOBs apoptosis was positively associated with the concentration of vaspin (Fig. 1). TUNEL analysis was used as a second metric of apoptosis in serum-deprived hOBs. Our data confirms that serum deprivation significantly induced hOBs apoptosis (Fig. 2a), and that treatment with 100 ng/ml vaspin protected hOBs from apoptosis induced by serum deprivation (Fig. 2b) (AI: 30.90 ± 1.65 vs. 7.52 ± 0.32 %; Fig. 2c).

Effects of vaspin on Bcl-2 and Bax protein expression in hOBs

Western blot analysis was used to assess the effects of vaspin on the expression of the apoptosis-related proteins Bcl-2 and Bax in hOBs. The results showed that vaspin exposure increased Bcl-2 protein expression and decreased Bax protein expression in hOBs, in a dose-dependent manner. When the Bax/Bcl-2 ratio in control cells was set to 1, 100 ng/ml vaspin treatment decreased this ratio to 0.08 (Fig. 3).

Vaspin activates ERK signaling pathway in hOBs

Western blot analysis was used to determine the effect of vaspin on intracellular signaling pathways, demonstrating that 100 ng/ml vaspin induced the phosphorylation of ERK after 5 min of stimulation, and the level of phosphorylation continued to increase until a peak was reached at 30 min (Fig. 4a). Conversely, vaspin had no effect on p38, JNK or PI3-K/Akt phosphorylation in hOBs (Fig. 4b). The phosphorylation of ERK by vaspin was abolished by the vaspin inhibitor PD98059 (Fig. 4c). Taken together, these results indicate that vaspin activates the MAPK/ERK signaling pathway in hOBs.

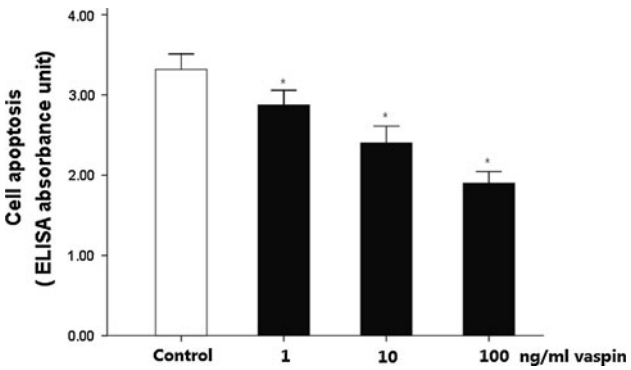

Fig. 1 Effect of vaspin on serum deprivation-induced hOBs apoptosis detected by cell death detection ELISA. Osteoblasts were deprived of serum for 24 h, then treated with vaspin (0, 1, 10, 100 ng/ml) for 48 h. ELISA results showed that vaspin suppressed serum deprivation-induced apoptosis in hOBs in a dose-dependent manner. Data are presented as the mean ± SD; $n = 5$; *$p < 0.05$ versus control

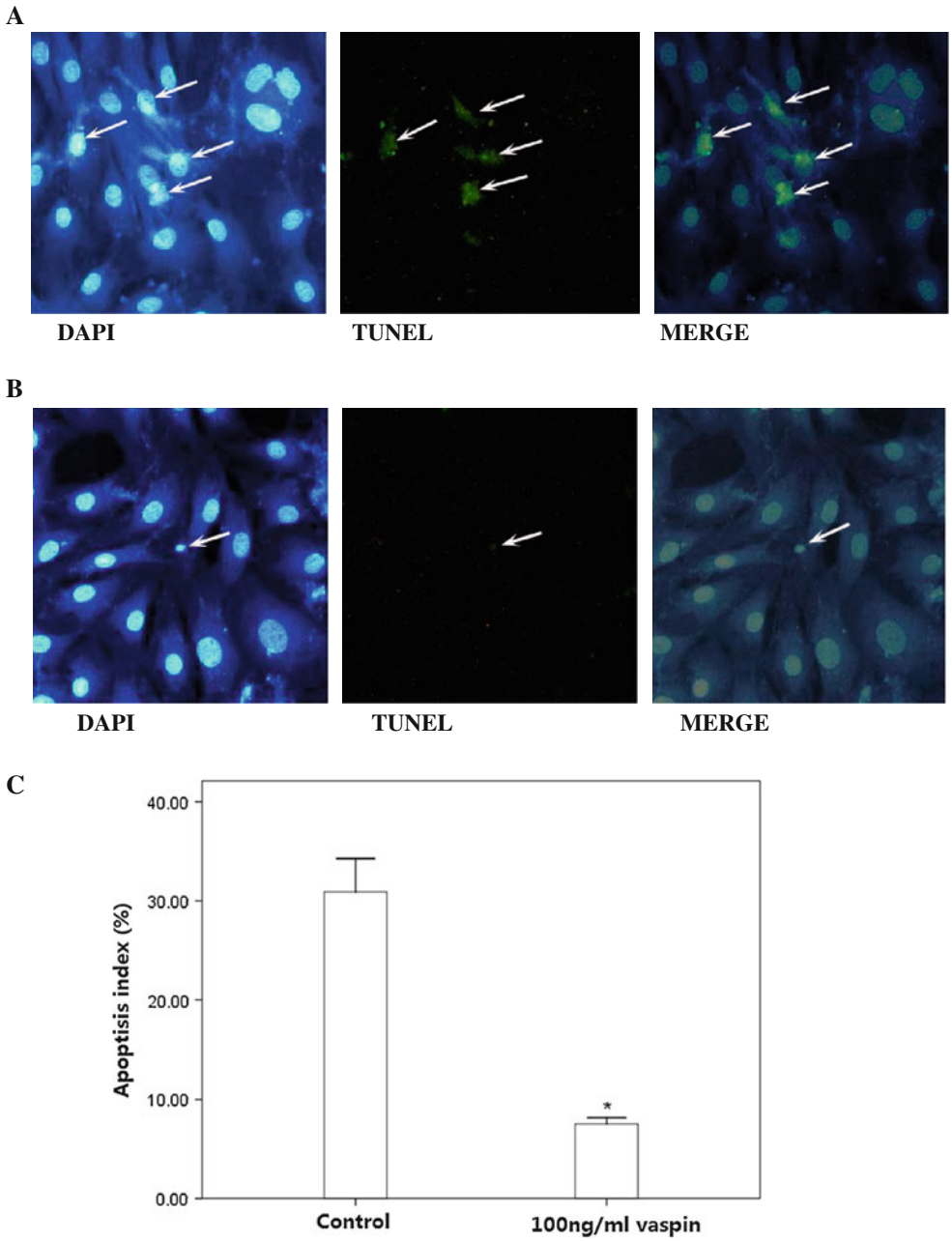

Fig. 2 Effect of vaspin on apoptosis of hOBs detected by TUNEL analysis. Osteoblasts were deprived of serum for 24 h, then treated with absence or precence of 100 ng/ml vaspin for 48 h. Representative images of TUNEL stained cells are shown for the control group (**a**) and cells were treated with 100 ng/ml vaspin for 48 h (**b**). Apoptotic hOBs are indicated by *white arrows*. The apoptosis index for control and vaspin-treated hOBs is shown (**c**). Original magnification of all images, ×400. Data are presented as the mean ± SD; $n = 4$; $^{**}p < 0.01$ versus control

ERK signaling mediates the anti-apoptotic effect of vaspin in hOBs

After demonstrating that vaspin activated the ERK signaling pathway in hOBs, we used an ELISA to determine if the ERK signaling pathway participates in regulating hOBs apoptosis. Our results show that the anti-apoptotic effect of vaspin exposure was reversed by pretreatment with PD98059 (Fig. 5), demonstrating that the anti-apoptotic role of vaspin in hOBs is mediated via the MAPK/ERK pathway.

Discussion

It is believed that osteoblast apoptosis plays an important role in embryogenesis and adult skeleton formation. Indeed,

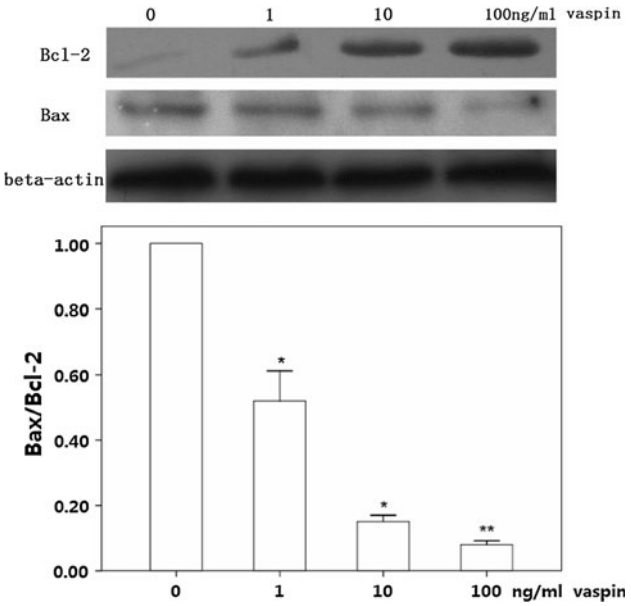

Fig. 3 Effect of vaspin on Bax and Bcl-2 protein expression in hOBs. Osteoblasts were deprived of serum for 24 h, then treated with 0–100 ng/ml vaspin for 48 h. Representative Western blots are shown for Bax, Bcl-2 and β-actin. The expression of Bax and Bcl-2 proteins was determined by densitometry of Western blot. $n = 3$; $^{*}p < 0.05$ and $^{**}p < 0.01$ versus control

apoptosis is recognized as a pivotal component of physiological bone turnover, repair, and regeneration (Bran et al. 2008). Multiple adipocytokines induce apoptosis by activating intracellular signaling pathways. For example, leptin exerts effects on apoptosis through various signaling pathways such as ERK, PI3-K/Akt, Fas and JAK2 (Kim et al. 2003; Chen et al. 2011; Wang et al. 2012; Mattioli et al. 2009; Tanaka and Umesaki 2008;Chen et al. 2007). Furthermore, adiponectin inhibits glucotoxicity-induced apoptosis by the PI3-K/Akt and AMPK signaling pathways (Xiao et al. 2011; Lin et al. 2009), whereas visfatin has been shown to prevent macrophage apoptosis by activating the IL-6/STAT3 signaling pathway (Li et al. 2008). Ghrelin, a member of the brain-gut peptide family, has been shown to directly inhibit osteoblast apoptosis (Nikolopoulos et al. 2010). In our previous studies, we have demonstrated that apelin suppresses serum deprivation-induced apoptosis in hOBs (Xie et al. 2007). Apelin also protects rat bone marrow mesenchymal stem cells against apoptosis induced by serum deprivation, which has been linked to the MAPK/ERK and PI3-K/Akt signaling pathways (Zeng et al. 2012). Apelin also prevents human vascular smooth muscle cells from entering apoptosis through the APJ/PI3-K/Akt signaling pathway (Cui et al. 2010).

The role of vaspin in apoptosis and its underlying mechanism of action have not been studied previously in osteoblasts. However, vaspin has been shown to inhibit free fatty acid-induced apoptosis in vascular endothelial cells via the PI3-K/Akt pathway (Jung et al. 2011). In the present study, we investigated the effect and mechanism of vaspin on serum deprivation-induced apoptosis in hOBs.

According to the apoptosis measurement, there are several ways to determine the apoptosis level in cells. Apoptosis leads to internucleosomal cleavage of DNA and the generation of mono- and oligonucleosomes, which are tightly complexed with histones. The Cell Death Detection ELISA kit is based on the quantitative detection of histone-associated DNA fragments in mono- and oligonucleosomes (a marker for apoptotic cells), which is widely used in determining cell apoptosis. TUNEL is a classic method to evaluate the apoptosis through visualizing nuclei containing fragmented DNA by labeling the exposed termini of DNA. Using Cell Death ELISA and TUNEL assay, our results show that vaspin suppresses serum deprivation-induced apoptosis in hOBs in a dose-dependent manner.

Proteins involved in the regulation of apoptosis include several inhibitors, including Bcl-2 and Bcl-xl, as well as apoptosis inducers, including Bak, Bax, Bid and Bad. The Bcl-2 family plays vital role in regulating apoptosis. Apoptosis-inhibiting proteins include Bcl-2 and Bcl-xl, and apoptosis-inducing proteins include Bak, Bax, Bid and Bad. The occurrence of apoptosis is determined by the balance of apoptosis-inhibiting and apoptosis-inducing proteins (Korsmeyer 1995; Moriishi et al. 2011; Adams and Cory 1998; Nakamura et al. 2003). We found that vaspin upregulated the expression of Bcl-2 and reduced the expression of Bax in hOBs. We interpret these results to indicate that the Bcl-2 family is involved in regulation of the inhibition of hOBs apoptosis by vaspin.

To gain further insight into the mechanism of the capacity of vaspin to inhibit hOBs apoptosis, we investigated several intracellular signaling pathways. The MAPKs are serine/threonine kinases, including ERK, JNK, p38 and ERK5/BMK1 pathways. Previous research has demonstrated the involvement of many of these signaling pathways (e.g. ERK, Wnt, FGF, BMP, PKC and gp130) in the regulation of osteoblast apoptosis (Arbon et al. 2012; Tamura et al. 2010; Formigue et al. 2005; Almeida et al. 2010; Franchimont et al. 2005). Zhang et al. (2011) reported that taurine inhibited serum deprivation-induced osteoblast apoptosis by activating the taurine transporter/ERK signaling pathway. The present study investigated the effect of vaspin on MAPK and PI3-K/Akt pathways. Our results show that vaspin activates ERK, but does not activate p38, JNK or PI3-K/Akt in hOBs. Pretreatment with the ERK inhibitor PD98059 reversed the activation of ERK in hOBs, indicating that vaspin suppresses hOBs apoptosis by activating the MAPK/ERK signaling pathway. Jung et al. (2011) previously established that vaspin inhibits free fatty acid-induced vascular endothelial cell apoptosis by activating PI3-K/Akt pathway, indicating that vaspin may

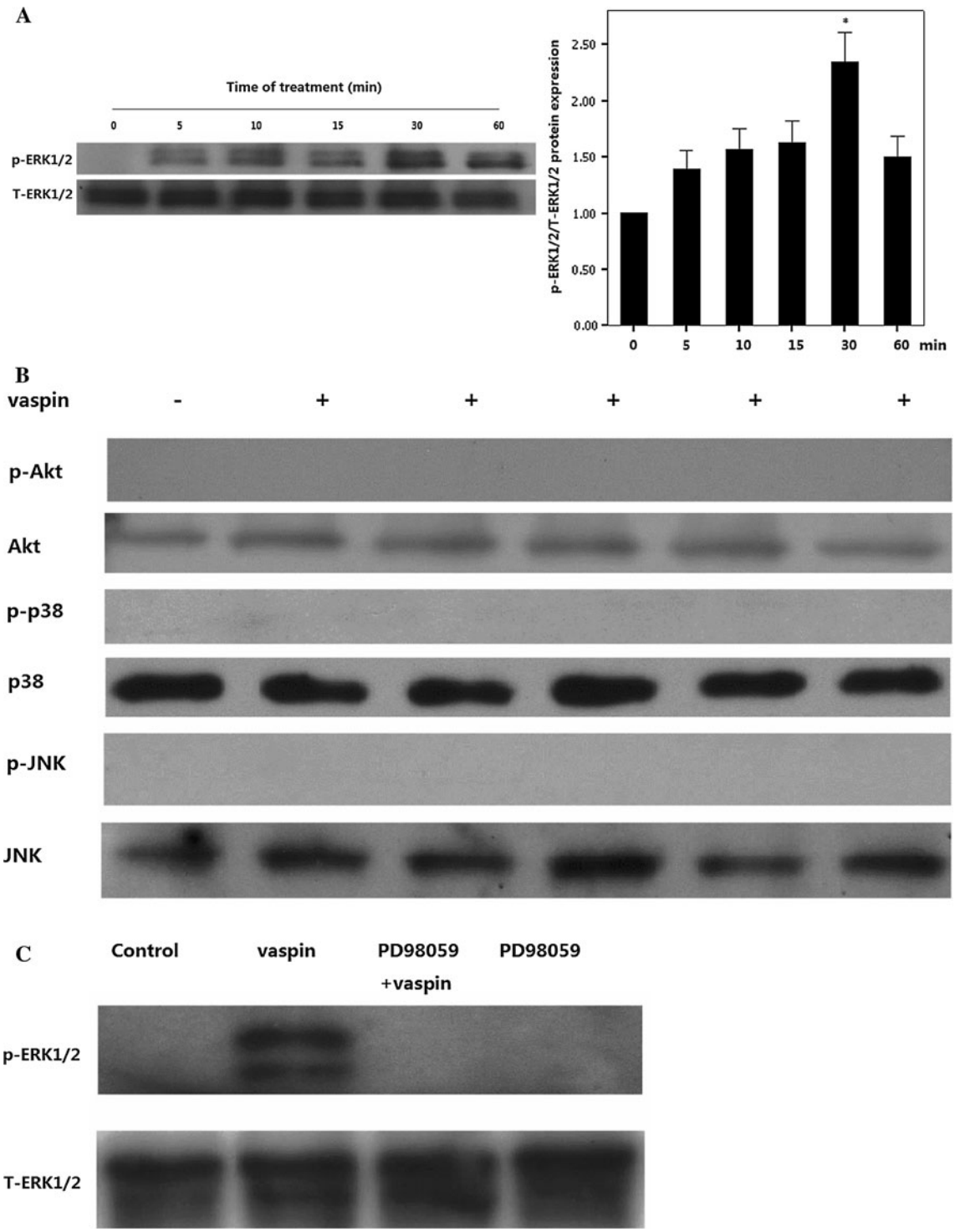

Fig. 4 Involvement of MAPK and PI3-K/Akt phosphorylation in vaspin-stimulated hOBs. Cell lysates were subjected to Western blotting and incubated with antibodies against p-ERK1/2, T-ERK1/2, p-P38, P38, p-JNK, JNK, p-Akt, and Akt. Representative results are shown. **a** hOBs were exposed to 100 ng/ml vaspin for 0–60 min to assess ERK activation. **b** hOBs were exposed to 100 ng/ml vaspin for 0–60 min to assess p-p38, p38, p-Akt, Akt, p-JNK and JNK activation. **c** hOBs were pretreated with 10 μM PD98059 for 3 h before exposure to 100 ng/ml vaspin for 30 min. $n = 3$; $^*p < 0.05$ versus 0 min

play an important role in inhibiting apoptosis. However, no activation of the PI3-K/Akt, p38 or JNK pathways was observed in osteoblasts in the present study. In human umbilical vein endothelial cells vaspin mediated only slight inhibition of TNF-α-induced activation of Akt, but not that of JNK, NF-κB or p38 (Fu et al. 2009). This indicates that,

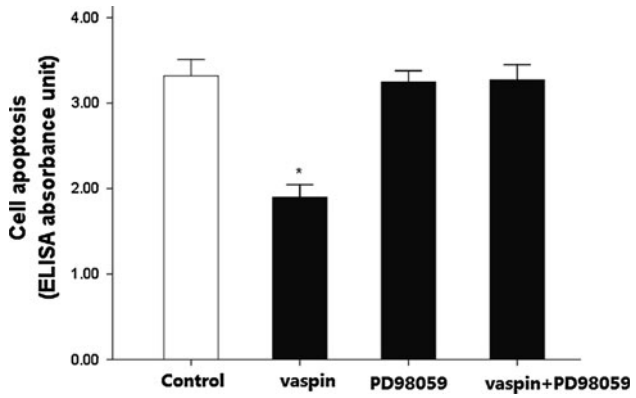

Fig. 5 The ERK signaling pathway mediates the capacity of vaspin to inhibit serum deprivation-induced apoptosis of hOBs. Osteoblasts were pretreated with 10 μM PD98059 for 3 h before exposure to 100 ng/ml vaspin for 48 h. Osteoblast apoptosis was analyzed by ELISA. Data are presented as the mean ± SD, $n = 5$, $^*p < 0.05$ versus control

as in the case of insulin and leptin, vaspin could have different targets affecting different signaling pathways in its various target cells.

In conclusion, our study demonstrates that vaspin protects hOBs from serum deprivation-induced apoptosis by activating the MAPK/ERK signal pathway, which suggests that vaspin is involved in bone metabolism through regulation of osteoblast apoptosis.

Acknowledgments This work was supported by funding from The National Natural Science Foundation of China (No. 81270962, 30801203, 30871191 and 81070246), science and technology grant of Development and Reform Commission of Hunan Province (2011–1318), education, science and technology grant of Department of finance of Hunan Province (2011–43).

Conflict of interest The authors declare no conflict of interest.

References

Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281:1322–1326

Almeida M, Han L, Ambrogini E et al (2010) Oxidative stress stimulates apoptosis and activates NF-kappaB in osteoblastic cells via a PKCbeta/p66shc signaling cascade: counter regulation by estrogen of androgens. Mol Endocrinol 24(10):2030–2037

Arbon KS, Christensen CM, Harvey WA et al (2012) Cadmium exposure activates the ERK signaling pathway leading to altered osteoblast gene expression and apoptotic death in Saos-2 cells. Food Chem Toxicol 50(2):198–205

Biver E, Salliot C, Combescure C et al (2011) Influence of adipokines and ghrelin on bone mineral density and fracture risk: a systematic review and meta-analysis. J Clin Endocrinol Metab 96(9):2703–2713

Bran GM, Stern-Straeter J, Hörmann K, Riedel F, Goessler UR (2008) Apoptosis in bone for tissue engineering. Arch Med Res 39(5):467–482

Brunetti L, Di Nisio C, Recinella L et al (2011) Effects of vaspin, chemerin and omentin-1 on feeding behavior and hypothalamic peptide gene expression in the rat. Peptides 32(9):1866–1871

Cao JJ (2011) Effects of obesity on bone metabolism. J Orthop Surg Res 15(6):30

Chen C, Chang YC, Liu CL et al (2007) Leptin induces proliferation and anti-apoptosis in human hepatocarcinoma cells by up-regulating cyclin D1 and down-regulating Bax via a Janus kinase 2-linked pathway. Endocr Relat Cancer 14(2):513–529

Chen JR, Lazarenko OP, Wu X et al (2010) Obesity reduces bone density associated with activation of PPARγ and suppression of Wnt/β-Catenin in rapidly growing male rats. PLoS One 5(10):e13704

Chen YC, Chen CH, Hsu YH et al (2011) Leptin reduces gentamicin-induced apoptosis in rat renal tubular cells via the PI3 K-Akt signaling pathway. Eur J Pharmacol 658(2–3):213–218

Cui RR, Mao DA, Yi L et al (2010) Apelin suppresses apoptosis of human vascular smooth muscle cells via APJ/PI3-K/Akt signaling pathways. Amino Acids 39(5):1193–1200

Formigue O, Mordrowski D, Marie PJ (2005) Apoptosis in membranous bone formation: role of fibroblast growth factor and bone morphogenetic protein signaling. Crit Rev Eukaryot Gene Expr 15(1):75–92

Franchimont N, Wertz S, Malaise M (2005) Interleukin-6: an osteotropic factor influencing bone formation? Bone 37(5):601–606

Fu BD, Yamawaki H, Okada M et al (2009) Vaspin can not inhibit TNF-alpha-induced inflammation of human umbilical vein endothelial cells. J Vet Med Sci 71(9):1201–1207

Gulcelik NE, Karakaya J, Gedik A et al (2009) Serum vaspin levels in type 2 diabetic women in relation to microvascular complications. Eur J Endocrinol 160:65–70

Hamed EA, Zakary MM, Ahmed NS et al (2011) Circulating leptin and insulin in obese patients with and without type 2 diabetes mellitus: relation to ghrelin and oxidative stress. Diabetes Res Clin Pract 94(3):434–441

Hida K, Wada J, Eguchi J et al (2005) Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci USA 102(30):10610–10615

Holecki M, Wiecek A (2010) Relationship between body fat mass and bone metabolism. Pol Arch Med Wewn 120(9):361–367

Jung CH, Lee WJ, Hwang JY et al (2011) Vaspin protects vascular endothelial cells against free fatty acid-induced apoptosis through a phosphatidylinositol 3-kinase/Akt pathway. Biochem Biophys Res Commun 413:264–269

Kim GS, Hong JS, Kim SW et al (2003) Leptin induces apoptosis via ERK/cPLA2/cytochrome pathway in human bone marrow stromal cells. J Biol Chem 278(24):21920–21929

Klöting N, Kovacs P, Kern M et al (2011) Central vaspin administration acutely reduces food intake and has sustained blood glucose-lowering effects. Diabetologia 54(7):1819–1823

Korsmeyer SJ (1995) Regulators of cell death. Trends Genet 11:101–105

Kitamura T, Itoh M, Noda T et al (2004) Combined effects of cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors on intenstinal tumorigenesis in adenomatous polyposis coli gene knockout mice. Int J Cancer 109(4):576–580

Lee YH, Magkos F, Mantzoros CS et al (2011) Effects of leptin and adiponectin on pancreatic β-cell function. Metabolism 60(12): 1664–1672

Li Y, Zhang Y, Dorweiler B et al (2008) Extracellular Nampt promotes macrophage survival via a nonenzymatic interleukin-6/STAT3 signaling mechanism. J Biol Chem 283(50):34833–34843

Lin P, Chen L, Li D et al (2009) Adiponectin reduces glucotoxicity-induced apoptosis of INS-1 rat insulin-secreting cells on a microfluidic chip. Tohoku J Exp Med 217(1):59–65

Liu YS, Lu Y, Liu W et al (2010) Connective tissue growth factor is a downstream mediator for preptin-induced proliferation and differentiation in human osteoblasts. Amino Acids 38(3):763–769

Mattioli B, Giordani L, Quarata MG et al (2009) Leptin exerts an anti-apoptotic effect on human dendritic cells via the PI3 K-Akt signaling pathway. FEBS Lett 583(7):1102–1106

Mohiti J, Talebi F, Afkhami-Ardekani M (2009) Circulation free leptin in diabetic patients and its correlation to insulin level. Pak J Biol Sci 12(4):397–400

Moriishi T, Maruyama Z, Fukuyama R et al (2011) Overexpression of Bcl-2 in osteoblasts inhibits osteoblast differentiation and induces osteocyte apoptosis. PLoS One 6(11):e27487

Nakamura H, Kumei Y, Morita S et al (2003) Antagonism between apoptotic (Bax/Bcl-2) and anti-apoptotic (IAP) signals in human osteoblastic cells under vector-averaged gravity condition. Ann N Y Acad Sci 1010:143–147

Nikolopoulos D, Theocharis S, Kouraklis G (2010) Ghrelin, another factor affecting bone metabolism. Med Sci Monit 16(7):RA147–RA162

Phalitakul S, Okada M, Hara Y et al (2011) Vaspin prevents TNF-α-induced intracellular adhesion molecular-1 via inhibiting reactive oxygen species-dependent NF-κB and PKCθ activation in cultured rat vascular smooth muscle cells. Pharmacol Res 64(5):493–500

Tamura M, Nemoto E, Sato MM et al (2010) Role of the Wnt signaling pathway in bone and tooth. Front Biosci(Elite Ed) 2:1405–1413

Tan BL, Heutling D, Chen J et al (2008) Metformin decreases the adipokine vaspin in overweight women with polycystic ovary syndrome concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. Diabetes 57:1501–1507

Tanaka T, Umesaki N (2008) Leptin regulates the proliferation and apoptosis of human endometrial epithelial cells. Int J Mol Med 22(5):683–689

Wada J (2008) Vaspin: a novel serpin with insulin-sensitizing effects. Expert Opin Investig Drugs 17(3):327–333

Wang D, Chen J, Chen H et al (2012) Leptin regulates proliferation and apoptosis of colorectal carcinoma through PI3K/Akt/mTOR signaling pathway. J Biosci 37(1):91–101

Xiao X, Dong Y, Zhong J et al (2011) Adiponectin protects endothelial cells from the damages induced by the intermittent high level of glucose. Endocrine 40(3):386–393

Xie H, Tang SY, Cui RR et al (2006) Apelin and its receptor are expressed in human osteoblasts. Regul Pept 134(2–3):118–125

Xie H, Yuan LQ, Luo XH et al (2007) Apelin suppresses apoptosis of human osteoblasts. Apoptosis 12(1):247–254

Yuan LQ, Liu YS, Luo XH et al (2007) Recombinant tissue metalloproteinase inhibitor-3 protein induces apoptosis of murine osteoblast MC3T3-E1. Amino Acids 35:123–127

Yuan LQ, Liu W, Cui RR et al (2010) Taurine inhibits osteoclastogenesis through the taurine transporter. Amino Acids 39(1): 89–99

Zaidi M, Buettner C, Sun L et al (2012) Minireview: the link between fat and bone: does mass beget mass? Endocrinology 153(5): 2070–2075

Zeng X, Yu SP, Taylor T et al (2012) Protective effect of apelin on cultured rat bone marrow mesenchymal stem cells against apoptosis. Stem Cell Res 8(3):357–367

Zhang LY, Zhou YY, Chen F et al (2011) Taurine inhibits serum deprivation-induced osteoblast apoptosis via the taurine transporter/ERK signaling pathway. Braz J Med Biol Res 44(7): 618–623