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ORIGINAL ARTICLE

Taurine restores Axl/Gas6 expression in vascular smooth muscle cell calcification model

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Abstract Our previous studies demonstrated that taurine inhibits osteoblastic differentiation of vascular smooth muscular cells (VSMCs) via the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway, but the underlying mechanism is not elucidated. The tyrosine kinase receptor Axl and its ligand growth arrest-specific protein 6 (Gas6) are expressed in VSMCs. Axl/Gas6 signaling system is known to inhibit VSMCs calcification. We herein showed that taurine partially restored Axl and Gas6 expression in β-glycerophosphate (β-GP)-induced VSMC calcification model. Taurine also induced activation of ERK, but not other two MAPKs including c-jun N-terminal Kinase (JNK) and p38 in VSMCs. Either knockdown of the taurine transporter (TAUT) or treatment with the ERK-specific inhibitor PD98059 blocked the activation of ERK by taurine and abolished taurine-induced Axl/Gas6 expression and calcium deposition reduction in β-GP-induced VSMC calcification model. These results demonstrate for the first time that taurine stimulates expression of Axl and Gas6 via TAUT/ERK signaling pathway in β-GP-induced VSMC calcification model.

Keywords Taurine · Vascular smooth muscular cells · Extracellular signal-regulated kinase · Axl · Gas6

Introduction

Vascular calcification has severe clinical consequences in a number of diseases including atherosclerosis, diabetes, and end-stage renal disease, and it is now considered an independent prognostic indicator of future adverse cardiovascular events. Moreover, vascular calcification is now recognized as a highly regulated process which is similar in many ways to bone mineralization. Osteoblast-like phenotypes transformation of vascular smooth muscular cells (VSMCs) is considered to be responsible for the formation of vascular calcification (Johnson et al. 2006; Guzman 2007; Demer and Tintut 2008).

Axl, a membrane receptor tyrosine kinase, is ubiquitously expressed, having been detected in a wide variety of organs and cells (Hafizi and Dahlbäck 2006a, b). Axl autophosphorylation can be induced by receptor dimerization in response to binding of its ligand growth arrest-specific protein 6 (Gas6), a vitamin K-dependent protein. The Axl/Gas6 signaling system is now emerging as an important regulator of mammalian physiology and pathology with distinct and diverse mechanisms of action, depending on the cell type or organ system involved

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(Hafizi and Dahlbäck 2006a, b; Sasaki et al. 2006). In VSMCs, Axl/Gas6 signaling induces VSMC migration in vitro and in vivo (Korshunov et al. 2006). In addition, Axl contributes to generation of reactive oxygen species in VSMCs (Konishi et al. 2004). More importantly, the restoration of Axl/Gas6 signaling strongly inhibits apoptosis and calcification of VSMCs, and deficiency of Axl/Gas6 signaling promotes mineralization of VSMCs (Nakano et al. 1996; Collett et al. 2007; Melaragno et al. 2004; Son et al. 2006, 2007).

Taurine is a sulfur-containing β-amino acid that exists in free form in mammals. Various physiological roles have been suggested for taurine, including calcium modulation, membrane stabilization, intracellular osmosis regulation, and regulation of protein phosphorylation (Oja and Saransaari 2007). Taurine is necessary for normal development, and its deficiency leads to defects in growth, tissue differentiation, and immune development. A worldwide epidemiological study revealed a strong inverse association between population levels of urinary taurine excretion and ischemic heart disease mortality, suggesting that taurine intake could be effective in preventing cardiovascular disease (Yamori et al. 2001). Murakami et al. (2002) showed that taurine prevented the progression of atherosclerosis in rabbits, indicating its probable therapeutic role in atherosclerosis. Moreover, our previous study demonstrated that taurine transporter (TAUT) was expressed in VSMCs and taurine prevented β-glycerophosphate (β-GP)-induced calcification in these cells through ERK signaling pathway (Liao et al. 2007, 2008). The present study was undertaken to investigate the action of taurine on Axl and Gas6 expression in β-GP-induced VSMC calcification model and to further determine whether the TAUT/ERK signaling pathway is involved in this action.

Materials and methods

Reagents

Taurine and β-GP were purchased from Sigma (St. Louis, MO, USA). Anti-Axl and anti-Gas6 polyclonal antibodies were purchased from Santa Cruz Biotechnology Inc., (Waltham, MA, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse and anti-rabit IgGs were also purchased from Santa Cruz Biotechnology Inc. Monoclonal anti-β-actin antibody was purchased from Cell Signaling Inc., (Danvers, MA, USA). Male Sprague–Dawley rats (weighing 200–250 g) were purchased from the Animal Center, the Second Xiangya Hospital of Central South University. All animal experiments in this study were performed with the approval of the Animal Care Committee of the Second Xiangya Hospital of Central South University.

Cell culture

Rat VSMCs were acquired by an explant method described by Campbell et al. (Campbell and Campbell 1993). Briefly, a fragment of rat thoracic aorta was stripped of intima and adventitia. The remaining medial layer was cut into small pieces and placed in Dulbecco's Modified Eagle's medium, containing 4.5 g/l glucose, 10 mM sodium pyruvate, and 20% fetal bovine serum supplemented with 100 U/ml of penicillin and 100 μg/ml of streptomycin, at 37°C, in a humidified atmosphere containing 5% CO_2. After 5–7 days, cells migrated from the explants, and explant fragments were removed after 15 days of culture. VSMCs were passaged every 3–4 days, and experiments were performed using cultured primary cells with passage numbers between 3 and 6. The VSMC phenotype was confirmed by immunostaining the cells for smooth muscle α-actin.

Real-time quantitative RT-PCR assay for mRNA expression

Real-time quantitative RT-PCR analysis was done using Roche Molecular LightCycler (Roche Applied Science, Indianapolis, IN, USA) as described previously (Xie et al. 2007a), which is a combined thermal cycler and fluorescence detector with the ability to monitor the progress of individual PCR reactions optically during amplification. Total RNA from cultured cells was isolated using Trizol reagent (Gibco), and reverse transcription was performed using 1.0 μg total RNA and the reverse transcription kit purchased from Promega. Amplification reactions were set up in 25 μl reaction volumes containing amplification primers and SYBR Green PCR Master Mix (PE Applied Biosystems). 1 μl volume of cDNA was used in each amplification reaction. Preliminary experiments were carried out to optimize primer concentrations. The PCR primers were as follows: Axl sense, 5′-gacctagctgccaggaactg-3′; Axl antisense, 5′-acacgtcactcttgctggtg-3′; Gas6 sense, 5′-ttctgcttgtgcaaagatgg-3′; Gas6 antisense, 5′-ccccac aggtgtctgagtct-3′; β-actin sense, 5′-gtcgtaccactggcattgtg-3′, β-actin antisense, 5′-ctctcagctgtggtggtgaa-3′.

For each analysis, amplification and calibration curves were ran in parallel in triplicate. Each sample was analyzed three times and each experiment was repeated at least once. Amplification data were analyzed using the Sequence Detector System Software (PE Applied Biosystems). Relative quantification were calculated by normalizing the test crossing thresholds (Ct) with the β-actin amplified control

Ct. The results were normalized to β-actin and expressed as percentage of controls.

Quantification of calcium deposition

Cell monolayers were decalcified with 0.6 M HCl for 24 h. The calcium content was determined by measuring the concentrations of calcium in the HCl supernatant by atomic absorption spectroscopy. After decalcification, the cells were washed three times with phosphate buffered solution (PBS) and solubilized with 0.1 M NaOH/0.1% sodium dodecyl sulfate (SDS). The protein content was measured with a Bradford protein assay. The calcium content of the cell layer was normalized to protein content.

Cell apoptosis measurement

Apoptosis was assessed directly by measurement of cytoplasmic nucleosomes (i.e., DNA complexed with histone in the cytoplasm) using a Cell Death Detection ELISA Kit (Roche Diagnostics GmbH, Roche Molecular Biochemicals, Mannheim, Germany), according to the kit protocol. This Kit allows a specific determination of mono- and oligo-nucleosomes in the cytoplasmic fraction of cell lysates (Tang et al. 2007; Xie et al. 2007b, 2008, 2009; Wang et al. 2009). Briefly, the cell layers were rinsed with PBS and extracted with 0.5 ml of lysis buffer after 30 min incubation at 4°C. The cell lysates were then centrifuged for 10 min at 15,000 rpm, and aliquots of the supernatant were tested for apoptosis using the Cell Death Detection Kit.

Western blot analysis

Cells were harvested at 4°C and lysed on ice in lysis buffer containing 50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 1% Triton X-100, 0.02% sodium azide, 10 mM EDTA, 10 μg/ml aprotinin and 1 μg/ml aminoethylbenzenesulfonyl fluoride. To detect changes of MAPKs phosphorylation, treated cells were washed quickly with cold PBS containing 5 mM of EDTA and 0.1 mM of 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 (Xie et al. 2006). Total protein in cell lysates was quantified by the Bradford protein assay. Equal amount of protein (50 μg/lane) was subjected to SDS gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membranes were blocked for 1 h in 5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20 (TBS). The antibodies were then added in 5% milk and incubated for 2 h at room temperature. The membranes were then washed with TBS and incubated with appropriate HRP-conjugated secondary antibodies (1:2500, Santa Cruz) in TBS for 1 h at room temperature. The blots were then washed with TBS and visualized with the chemiluminescent detection method using the SuperSignal West Pico Substrate (Pierce, Rockford, USA).

RNA interference for TAUT

RNA interference was used to down-regulate the expression of TAUT in rat VSMCs. Short hairpin RNAs (shRNAs) of TAUT were synthesized by Genesil Biotechnology Co., (Wuhan, China). Target sense sequence: 5′-GATCC AGACTTCCACAAAGACATCTTCAAGAGAGATGTC TTT GTGGAAGTCTTTTTTTGGAAA-3′; antisense: 5′-A GCTTTTCCAAAAAAAGACTTCCACA AAGACATCT CTCTTGAAGATGTCTTTGTGGAAGTCTG-3′. The base pairs underlined in target sequences are the restriction sites BamHI and HindIII. The p*Silencer*^{TM} 2.1-U6 neo plasmid (Ambion Inc., Austin, TX, USA) was selected to express TAUT shRNAs. For gene knockdown experiments, VSMCs were plated in 6-well dishes and cultured for 24 h in medium without antibiotics and then transfected with shRNAs using Lipofectamine 2000 (Invitrogen Inc. Carlsbad, CA, USA) according to the manufacturer's instructions. After 24 h of culture, the transfection media were replaced with selection media containing 600 μg/ml G418. The resulting G418-resistant cell colonies were picked and expanded, then confirmed TAUT expression knockdown cell colonies were maintained in 300 μg/mL G418 media and used for further analysis.

Statistical analysis

SPSS 13.0 was used for the statistical analyses. The results were provided as mean ± SD. Comparisons were made using a one-way ANOVA. *P* values of less than 0.05 were considered statistically significant in all cases. All in vitro experiments were repeated at least three times, and representative experiments were shown.

Results

TAUT expression was knockdown by shRNA in VSMCs

Western blot analysis revealed that TAUT protein expression (70 kDa) could be detected in VSMCs (Fig. 1). This is consistent with our previous study demonstrated that TAUT was expressed in VSMCs (Liao et al. 2007). Treatment with shRNA-TAUT blocked the expression of

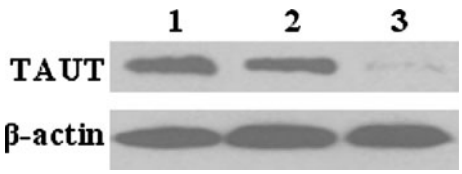

Fig. 1 Effects of shRNAs on TAUT protein expression in VSMCs. Total cellular protein was subjected to Western blot analysis. The membrane was first probed with anti-TAUT antibody which identified the bands at 70 kDa, then the bound first antibody was removed with a stripping SDS solution (2% SDS, 0.1 M mercaptoethanol, and 50 mM Tris–HCl, pH 7.0) and the membrane was re-probed with anti-β-actin antibody as a loading control, which identified the bands at 43 kDa. Shown are the representative results. *Lane 1* VSMCs, *lane 2* shRNA-control transfected VSMCs, *lane 3* shRNA-TAUT transfected VSMCs

TAUT protein in VSMCs since there were no bands detected (Fig. 1).

Taurine restores Axl and Gas6 expression and inhibits cell apopotosis in VSMC calcification model

Real-time quantitative RT-PCR analysis showed that treatment with 5–20 mM taurine for 24 h stimulates Axl and Gas6 mRNA expression levels in VSMCs (Fig. 2a), and treatment with 5–20 mM taurine for 6, 9 or 12 days partially reversed the β-GP-induced decrease in the transcription of Axl and Gas6 in VSMCs (Fig. 2b). In addition, treatment with 5–20 mM taurine for 24 h also increased Axl and Gas6 protein expression levels in VSMCs (Fig. 3).

After 12 day of culture, the amount of calcium deposition in the β-GP-treated VSMC group dramatically increased compared to the vehicle-treated group, while co-incubation with 5–20 mM taurine markedly decreased the induced calcium deposition (Fig. 4a); likewise, the apoptosis in the β-GP-treated VSMCs markedly increased compared to the vehicle-treated cells, while co-incubation with 5–20 mM taurine also markedly decreased the induced cell apoptosis (Fig. 4b); conversely, the relative expression levels of both Axl and Gas6 proteins in the β-GP-treated VSMCs significantly decreased compared to those in the vehicle-treated cells, and co-incubation with 5–20 mM taurine partially restored their expression levels (Fig. 4c).

Taurine stimulates ERK1/2 activation in VSMCs via TAUT

We examined whether taurine-induced MAPKs signaling in VSMCs. Taurine at 20 mM concentration has no effect on the activities of JNK and p38, and none of their phosphorylated forms were detected (Fig. 5a). Taurine increased phosphorylated ERK1/2 levels within 5 min of incubation. The peak activation of ERK occurred at 15 min.

Figure 5b shows that the activation of ERK1/2 by taurine was inhibited by the specific ERK inhibitor, PD98059. Suppression of TAUT with shRNA also blocked the activation of ERK by taurine. These data indicate that taurine activates the ERK signaling pathway via TAUT in VSMCs.

Fig. 2 Effect of taurine on Axl and Gas6 mRNA expression in short-period cultured VSMCs and long-period cultured β-GP-induced VSMC calcification model. **a** RNA was isolated from cells cultured in the serum-free media and treated with vehicle or different concentrations of taurine for 24 h. Expression was assessed by real-time quantitative RT-PCR and normalized to β-actin. Results are expressed as folds of vehicle-treated control cells. The *bars* represent the mean ± SD ($n = 3$; $^*P < 0.05$ vs. vehicle-treated control cells). **b** RNA was isolated from cells cultured in the presence of β-GP and treated with different concentrations of taurine for 6, 9, or 12 days. Vehicle-treated cells were used as control. Expression was assessed by real-time quantitative RT-PCR and normalized to β-actin. Results are expressed as percents of control cells. The *bars* represent the mean ± SD ($n = 3$; $^*P < 0.05$ vs. β-GP-treated control cells) ▶

TAUT/ERK signaling pathway mediates taurine-induced Axl and Gas6 expression and calcium deposition reduction in VSMC calcification model

We show that TAUT knockdown with shRNA blocked taurine-induced Axl and Gas6 protein expression and calcium deposition reduction in β-GP-induced VSMC calcification model (Fig. 6a, b). We also show that PD98059, a specific ERK inhibitor, abolished taurine-induced Axl and Gas6 protein expression and calcium deposition reduction in the same cell model (Fig. 6c, d). These data indicate that taurine restores Axl and Gas6 expression and inhibits calcium deposition in VSMC calcification model via TAUT/ERK signaling pathway.

Discussion

Our present data indicate that taurine not only stimulates both Axl and Gas6 expression in short-period cultured VSMCs, but also restores both Axl and Gas6 expression in long-period cultured β-GP-induced VSMC calcification model via TAUT/ERK signaling pathway.

Mineralization of soft tissues occurs under pathologic conditions and has detrimental consequences, particularly when it occurs in blood vessels. Calcification of arterial plaques decreases vessel elasticity, augments plaque brittleness, and increasingly leads to cerebral embolization during cardiac surgeries (Roach et al. 1996). It is, therefore, associated with an increased risk of myocardial infarction, neurocognitive impairment and perioperative death. Despite its clinical significance, the molecular mechanisms

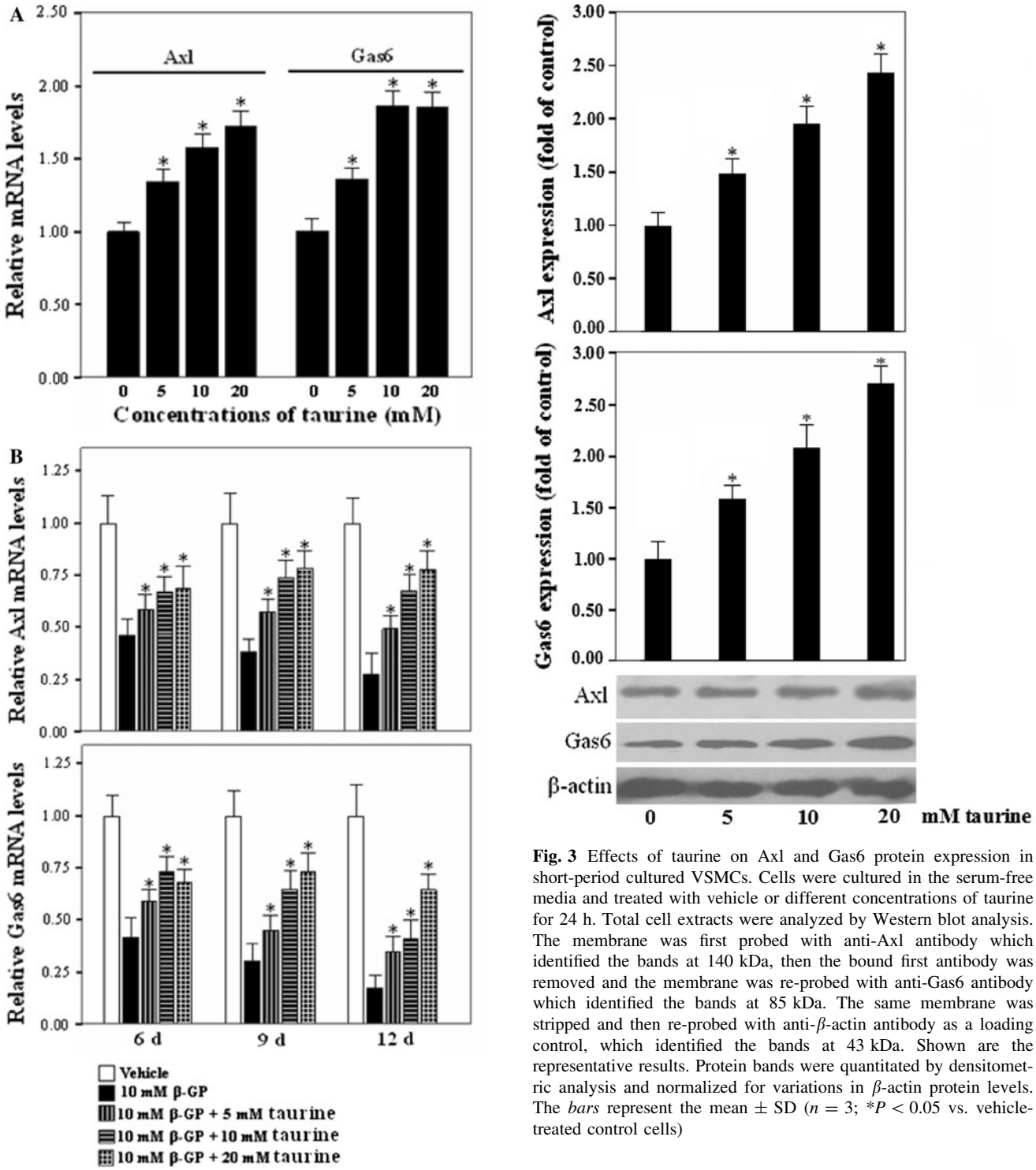

Fig. 3 Effects of taurine on Axl and Gas6 protein expression in short-period cultured VSMCs. Cells were cultured in the serum-free media and treated with vehicle or different concentrations of taurine for 24 h. Total cell extracts were analyzed by Western blot analysis. The membrane was first probed with anti-Axl antibody which identified the bands at 140 kDa, then the bound first antibody was removed and the membrane was re-probed with anti-Gas6 antibody which identified the bands at 85 kDa. The same membrane was stripped and then re-probed with anti-β-actin antibody as a loading control, which identified the bands at 43 kDa. Shown are the representative results. Protein bands were quantitated by densitometric analysis and normalized for variations in β-actin protein levels. The *bars* represent the mean ± SD ($n = 3$; $^*P < 0.05$ vs. vehicle-treated control cells)

underlying the regulation of vascular calcification are unclear. However, recent emerging evidence supports the idea that ectopic calcification, such as bone mineralization, is a cell-regulated process. Osteoblastic differentiation of VSMCs is considered to be responsible for the formation of vascular calcification (Johnson et al. 2006; Guzman 2007; Demer and Tintut 2008). Up-regulation of Axl/Gas6 signaling has recently been reported to inhibit osteoblastic differentiation of VSMCs (Son et al. 2006, 2007; Collett et al. 2007). Collett et al. (2007) demonstrated that signaling through Axl inhibited calcification of VSMCs in vitro. Son et al. (2006, 2007) reported that statins protected VSMCs from inorganic phosphate-induced calcification via restoration of the Axl/Gas6 pathway.

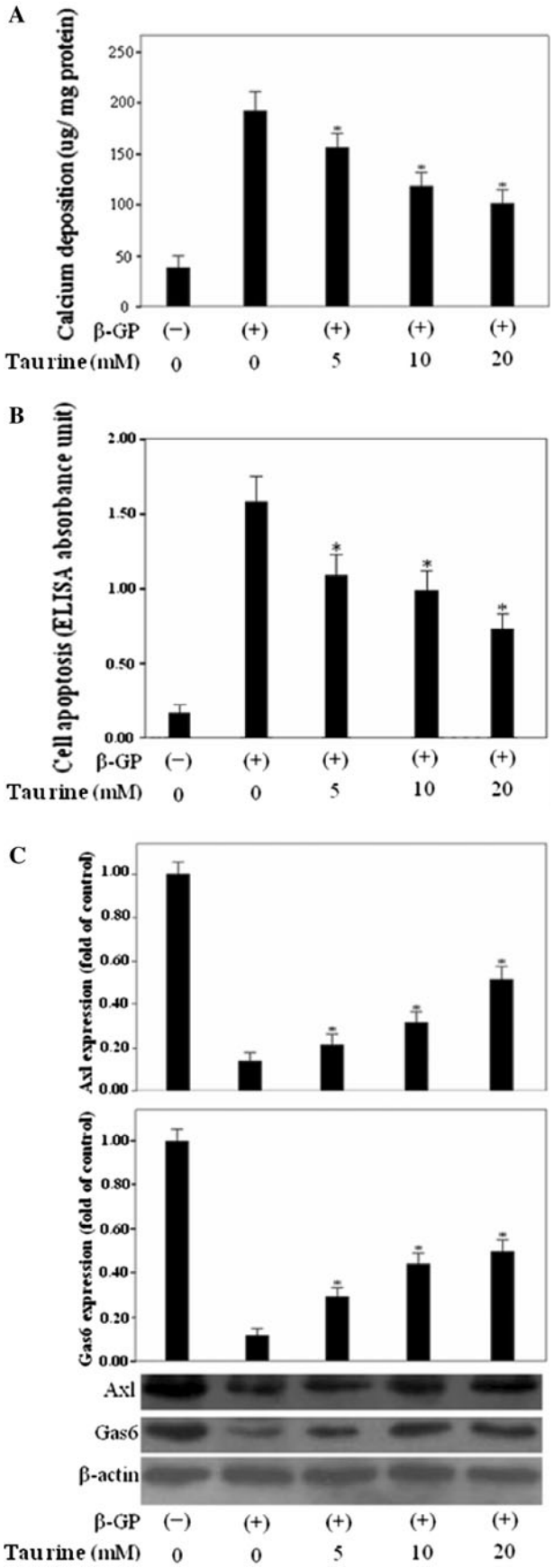

Fig. 4 Effects of taurine on calcium deposition, apoptosis and expression of Axl and Gas6 protein in long-term cultured β-GP-induce VSMC calcification model. Cells were cultured in the presence of β-GP and treated with different concentrations of taurine for 12 days. Cells treated with β-GP alone were used as control. **a** Effect of taurine on calcium deposition. The cell layer calcium content was determined as described in "Materials and methods" and presented as mean ± SD ($n = 4$; $^*P < 0.05$ vs. control cells). **b** Effect of taurine on cell apoptosis. Apoptosis was assessed using a Cell Death Detection Kit, and expressed as ELISA absorbance units. The *Bars* represent the mean ± SD ($n = 4$; $^*P < 0.05$ vs. control cells). **c** Effect of taurine on Axl and Gas6 protein expression. The total cellular protein was subjected to Western blot analysis as described under Fig. 3 legend. The *bars* represent the mean ± SD ($n = 3$; $^*P < 0.05$ vs. control cells)

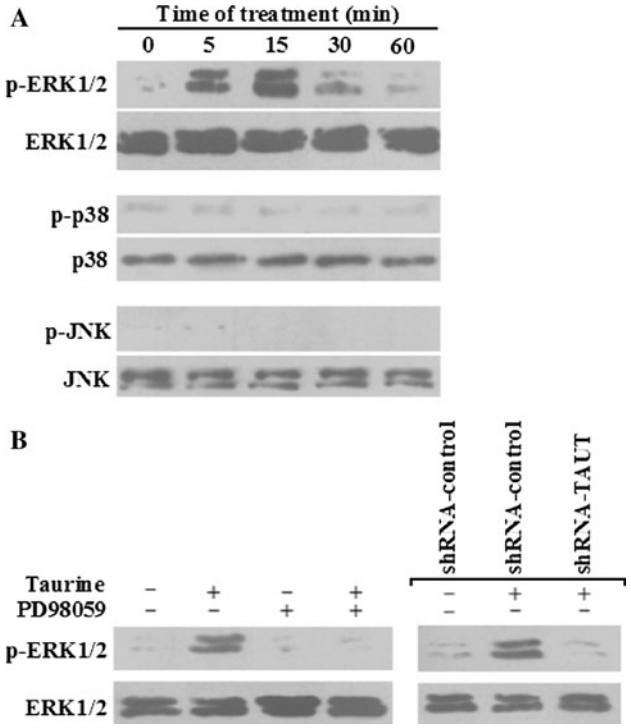

Fig. 5 Effect of taurine on MAPKs activation in VSMCs. Cell lysates were subjected to Western blot analysis using antibodies against p-ERK1/2, ERK1/2, p-JNK, JNK, p-p38 and p38. The representative results were shown. **a** Cells were treated with 20 mM taurine for 5–60 min. **b** Cells were treated with 10 μM PD98059 for 2 h prior to treatment with 20 mM taurine for 15 min. Both shRNA-control transfected cells and shRNA-TAUT transfected cells were treated with 20 mM taurine for 15 min

Taurine, one of the metabolites of methionine and cysteine, has been shown to have anti-hypertensive and anti-atherogenic effects in animal models. Epidemiological studies also revealed that taurine intake correlates inversely with the incidence of coronary heart disease (Yamori et al. 2001). The molecular mechanism underlying this protective effect is obscure, and some researchers have attributed this effect to its cholesterol-lowering properties

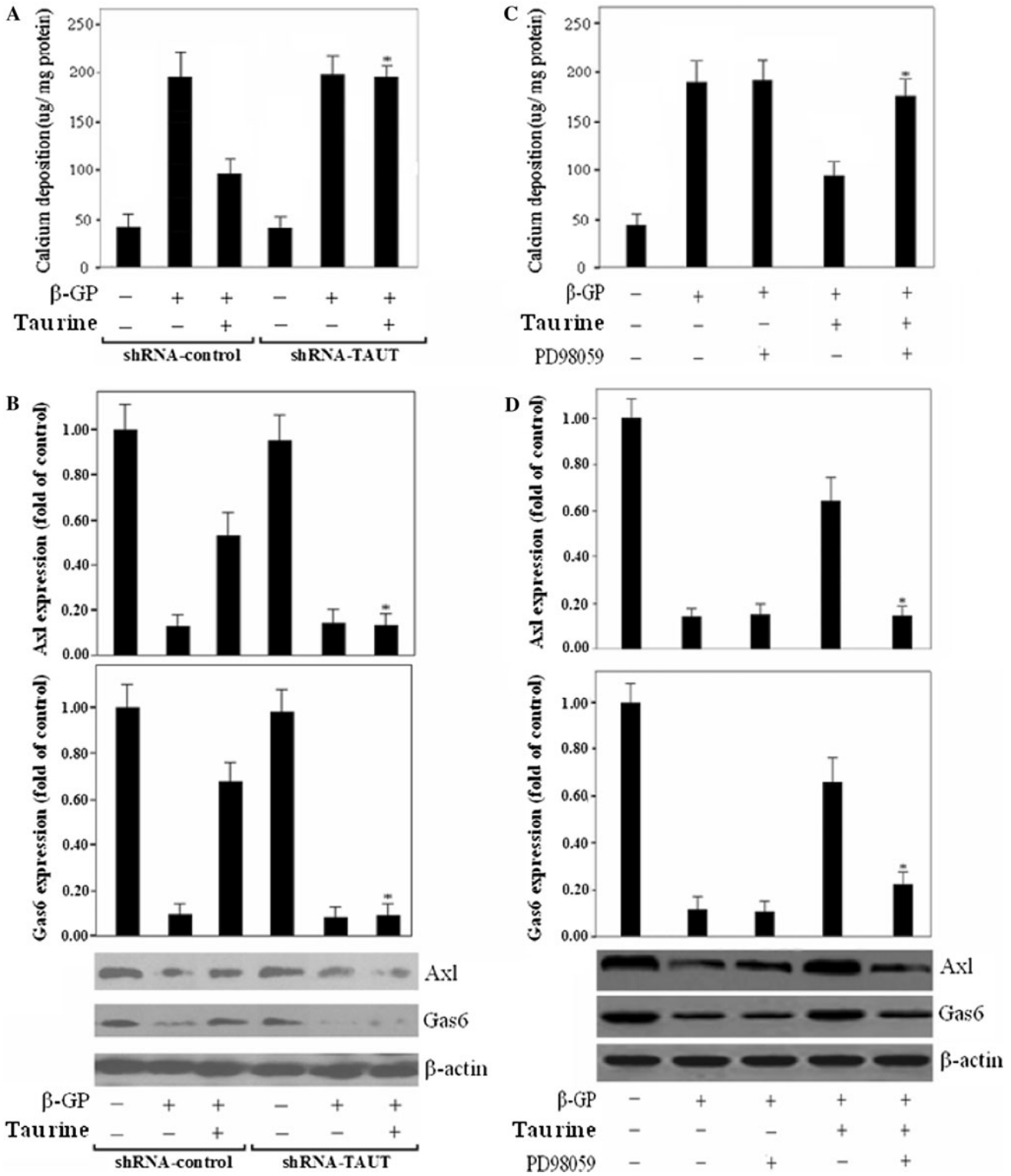

Fig. 6 TAUT/ERK signaling pathway mediates taurine-induced Axl and Gas6 protein expression in *β*-GP-induced VSMC calcification model. The cell layer calcium content was determined as described in "Materials and methods". Total cell extracts were analyzed by Western blot analysis as described under Fig. 3 legend. Shown are representative results. **a**, **b** Both shRNA-control transfected cells and shRNA-TAUT transfected cells were cultured in the absence or presence of *β*-GP and the *β*-GP-induced cells were also treated with vehicle or 20 mM taurine for 12 days. The *bars* represent the mean ± SD ($n = 3$; * $P < 0.05$ vs. *β*-GP-induced shRNA-control transfected cells with taurine treatment). **c**, **d** Cells were cultured in the absence or presence of *β*-GP and the *β*-GP-induced cells were also treated with vehicle or 20 mM taurine alone, 10 μM PD98059 alone or combination of taurine and PD98059 for 12 days. The *bars* represent the mean ± SD ($n = 3$; *$P < 0.05$ vs. *β*-GP-induced cells with taurine treatment)

(Murakami et al. 1999). However, others reported that taurine could prevent atherosclerosis without significantly affecting the serum cholesterol level (Murakami et al. 2002). Taurine and its analogs are reported to inhibit the proliferation of VSMCs (Zhang et al. 1999). It was further demonstrated that taurine suppresses the platelet-derived growth factor-BB-induced proliferation of VSMCs (Yoshimura et al. 2005). Moreover, Li et al. (2004) demonstrated that taurine decreased the β-GP-induced calcification in VSMCs. Our previous studies showed that TAUT was expressed in VSMCs (Liao et al. 2007) and taurine could inhibit osteoblastic differentiation of VSMCs via ERK activation (Liao et al. 2008). Our present data show that treatment with β-GP can significantly inhibit Axl and Gas6 protein expression in VSMCs, a result that is consistent with previous study (Collett et al. 2007). More importantly, the present results show that taurine restores the expression levels of Axl and Gas6 in VSMCs.

Taurine is known to be transported by a specific transporter, the TAUT. In the intracellular space, taurine is present in millimolar concentrations, whereas taurine is found at the concentration of 20–100 μM in plasma (Huxtable 1992), suggesting that TAUT plays an important role in maintaining a high concentration of taurine in cells. Recently, a TAUT knockout mouse has been established (Heller-Stilb et al. 2002). The animals exhibited retinal degeneration and a marked impairment of reproduction. These phenomena suggest that TAUT is a functional protein in maintaining cell physiological function in vitro and in vivo. Our previous studies demonstrated that transcription and translation of TAUT occur in culture of VSMCs (Liao et al. 2007). Additionally, the TAUT expressed in VSMCs is a functional protein, which can take up [^{3}H]taurine (Liao et al. 2007). Our present study shows that taurine stimulates both Axl and Gas6 expression in VSMC calcification model through TAUT.

To gain further insight into the mechanisms by which taurine stimulates Axl and Gas6 expression, we examined intracellular signaling pathways. Here, we show that taurine induces activation of ERK1/2 but not p38 and JNK in VSMCs. Either knockdown of the TAUT or treatment with the ERK-specific inhibitor PD98059 blocks ERK1/2 activation by taurine and abolishes taurine-induced Axl/Gas6 expression and calcium deposition reduction in β-GP-induced VSMC calcification model. These data indicate that TAUT/ERK signaling pathway mediates taurine-induced Axl and Gas6 expression and calcium deposition reduction in VSMC calcification model.

ERK was involved in the osteoblastic differentiation and mineralization of VSMCs. However, the results were contradictory. All our present and previous data showed that taurine inhibited osteoblastic differentiation and mineralization of VSMCs via the ERK signaling pathway (Liao et al. 2008), which is consistent with a report by Radcliff et al. (2005), who demonstrated that insulin-like growth factor-I inhibited osteoblastic differentiation and vascular cell mineralization via both the ERK and PI3K pathways. However, Ding et al. (2006) demonstrated that fibronectin enhanced osteoblastic differentiation of VSMCs via the ERK signaling pathway. These studies do seem to suggest that the ERK pathway-mediated osteoblastic differentiation and mineralization of VSMCs is either inhibited or enhanced depending on the agent used in the study, therefore, a thorough and careful examination of the underlying mechanism is warranted to fully understand the involvement of the ERK pathway in this process. Our present study shows that taurine stimulates Gas6 and Axl expression in the β-GP-treated VSMCs via the ERK signaling pathway. Clearly, the results described in the present study suggest that the stimulation of expression of Axl and Gas6 might, at least partially, play a role in taurine-mediated inhibition of the osteoblastic differentiation and mineralization of VSMCs.

In conclusion, our present data provide the evidence that taurine restores Gas6 and Axl expression in VSMC calcification model through the TAUT/ERK signaling pathway, which may contribute to the inhibition of vascular calcification.

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References

Campbell JH, Campbell GR (1993) Culture techniques and their applications to studied of vascular smooth muscle. Clin Sci 85:501–512

Collett GD, Sage AP, Kirton JP, Alexander MY, Gilmore AP, Canfield AE (2007) Axl/phosphatidylinositol 3-kinase signaling inhibits mineral deposition by vascular smooth muscle cells. Circ Res 100:502–509

Demer LL, Tintut Y (2008) Vascular calcification: pathobiology of a multifaceted disease. Circulation 117:2938–2948

Ding HT, Wang CG, Zhang TL, Wang K (2006) Fibronectin enhances in vitro vascular calcification by promoting osteoblastic differentiation of vascular smooth muscle cells via ERK pathway. J Cell Biochem 99:1343–1352

Guzman RJ (2007) Clinical, cellular, and molecular aspects of arterial calcification. J Vasc Surg 45:A57–A63

Hafizi S, Dahlbäck B (2006a) Gas6 and protein S. Vitamin K-dependent ligands for the Axl receptor tyrosine kinase subfamily. FEBS J 273:5231–5244

Hafizi S, Dahlbäck B (2006b) Signalling and functional diversity within the Axl subfamily of receptor tyrosine kinases. Cytokine Growth Factor Rev 17:295–304

Heller-Stilb B, van Roeyen C, Rascher K, Hartwig HG, Huth A, Seeliger MW, Warskulat U, Haussinger D (2002) Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice. FASEB J 16:231–233

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72:101–163

Johnson RC, Leopold JA, Loscalzo J (2006) Vascular calcification: pathobiological mechanisms and clinical implications. Circ Res 99:1044–1059

Konishi A, Aizawa T, Mohan A, Korshunov VA, Berk BC (2004) Hydrogen peroxide activates the gas6-axl pathway in vascular smooth muscle cells. J Biol Chem 279:28766–28770

Korshunov VA, Mohan AM, Georger MA, Berk BC (2006) Axl, a receptor tyrosine kinase, mediates flow-induced vascular remodeling. Circ Res 98:1446–1452

Li J, Zhang B, Huang Z, Wang S, Tang C, Du J (2004) Taurine prevents beta-glycerophosphate-induced calcification in cultured rat vascular smooth muscle cells. Heart Vessels 19:125–131

Liao XB, Zhou XM, Li JM, Tan ZP, Liu LM, Zhang W, Tan H, Lu Y, Yuan LQ (2007) Taurine transporter is expressed in vascular smooth muscle cells. Amino Acids 33:639–643

Liao XB, Zhou XM, Li JM, Yang JF, Tan ZP, Hu ZW, Liu W, Lu Y, Yuan LQ (2008) Taurine inhibits osteoblastic differentiation of vascular smooth muscle cells via the ERK pathway. Amino Acids 34:525–530

Melaragno MG, Cavet ME, Yan C, Tai LK, Jin ZG, Haendeler J, Berk BC (2004) Gas6 inhibits apoptosis in vascular smooth muscle: role of Axl kinase and Akt. J Mol Cell Cardiol 37:881–887

Murakami S, Kondo-Ohta Y, Tomisawa K (1999) Improvement in cholesterol metabolism in mice given chronic treatment of taurine and fed a high-fat diet. Life Sci 64:83–91

Murakami S, Kondo Y, Sakurai T, Kitajima H, Nagate T (2002) Taurine suppresses development of atherosclerosis in Watanab heritable hyperlipidemic (WHHL) rabbits. Atherosclerosis 163:79–87

Nakano T, Kawamoto K, Higashino K, Arita H (1996) Prevention of growth arrest-induced cell death of vascular smooth muscle cells by a product of growth arrest-specific gene, Gas6. FEBS Lett 387:78–80

Oja SS, Saransaari P (2007) Pharmacology of taurine. Proc West Pharmacol Soc 50:8–15

Radcliff K, Tang TB, Lim J, Zhang Z, Abedin M, Demer LL, Tintut Y (2005) Insulin-like growth factor-I regulates proliferation and osteoblastic differentiation of calcifying vascular cells via extracellular signal-regulated protein kinase and phosphatidylinositol 3-kinase pathways. Circ Res 96:398–400

Roach GW, Kanchuger M, Mangano CM, Newman M, Nussmeier N, Wolman R, Aggarwal A, Marschall K, Graham SH, Ley C (1996) Adverse cerebral outcomes after coronary bypass surgery. Multicenter Study of Perioperative Ischemia Research Group and the Ischemia Research and Education Foundation Investigators. N Engl J Med 335:1857–1863

Sasaki T, Knyazev PG, Clout NJ, Cheburkin Y, Gohring W, Ullrich A, Timpl R, Hohenester E (2006) Structural basis for Gas6-Axl signalling. EMBO J 25:80–87

Son BK, Kozaki K, Iijima K, Eto M, Kojima T, Ota H, Senda Y, Maemura K, Nakano T, Akishita M, Ouchi Y (2006) Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring Gas6-Axl survival pathway. Circ Res 98:1024–1031

Son BK, Kozaki K, Iijima K, Eto M, Nakano T, Akishita M, Ouchi Y (2007) Gas6/Axl-PI3K/Akt pathway plays a central role in the effect of statins on inorganic phosphate-induced calcification of vascular smooth muscle cells. Eur J Pharmacol 556:1–8

Tang SY, Xie H, Yuan LQ, Luo XH, Huang J, Cui RR, Zhou HD, Wu XP, Liao EY (2007) Apelin stimulates proliferation and suppresses apoptosis of mouse osteoblastic cell line MC3T3–E1 via JNK and PI3-K/Akt signaling pathways. Peptides 28:708–718

Wang QP, Xie H, Yuan LQ, Luo XH, Li H, Wang D, Meng P, Liao EY (2009) Effect of progesterone on apoptosis of murine MC3T3–E1 osteoblastic cells. Amino Acids 36:57–63

Xie H, Tang SY, Cui RR, Huang J, Ren XH, Yuan LQ, Lu Y, Yang M, Zhou HD, Wu XP, Luo XH, Liao EY (2006) Apelin and its receptor are expressed in human osteoblasts. Regul Pept 134:118–125

Xie H, Tang SY, Luo XH, Huang J, Cui RR, Yuan LQ, Zhou HD, Wu XP, Liao EY (2007a) Insulin-like effects of visfatin on human osteoblasts. Calcif Tissue Int 80:201–210

Xie H, Yuan LQ, Luo XH, Huang J, Cui RR, Guo LJ, Zhou HD, Wu XP, Liao EY (2007b) Apelin suppresses apoptosis of human osteoblasts. Apoptosis 12:247–254

Xie H, Tang SY, Li H, Luo XH, Yuan LQ, Wang D, Liao EY (2008) L-carnitine protects against apoptosis of murine MC3T3–E1 osteoblastic cells. Amino Acids 35:419–423

Xie H, Tang LL, Luo XH, Wu XY, Wu XP, Zhou HD, Yuan LQ, Liao EY (2009) Suppressive effect of dexamethasone on TIMP-1 production involves murine osteoblastic MC3T3-E1 cell apoptosis. Amino Acids [Epub ahead of print]

Yamori Y, Liu L, Ikeda K, Miura A, Mizushima S, Miki T, Nara Y, WHO-Cardiovascular Disease Alimentary Comprarison (CARDIAC) Study Group (2001) Distribution of twenty-four hour urinary taurine excretion and association with ischemic heart disease mortality in 24 populations of 16 countries: results from the WHO-CARDIAC study. Hypertens Res 24:453–457

Yoshimura H, Nariai Y, Terashima M, Mitani T, Tanigawa Y (2005) Taurine suppresses platelet-derived growth factor (PDGF) BB-induced PDGF-beta receptor phosphorylation by protein tyrosine phosphatase-mediated dephosphorylation in vascular smooth muscle cells. Biochim Biophys Acta 1745:350–360

Zhang X, Tenner TE Jr, Lombardini JB (1999) Inhibition of rat vascular smooth muscle cell proliferation by taurine and taurine analogues. Biochem Pharmacol 57:1331–1339