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ORIGINAL ARTICLE

Taurine suppresses osteoblastic differentiation of aortic valve interstitial cells induced by beta-glycerophosphate disodium, dexamethasone and ascorbic acid via the ERK pathway

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Abstract Aortic valve calcification (AVC) is an active process characterized by osteoblastic differentiation of the aortic valve interstitial cells (AVICs). Taurine is a free β-amino acid and plays important physiological roles including protective effect of cardiovascular events. To evaluate the possible role of taurine in AVC, we isolated human AVICs from patients with type A dissection without leaflet disease. We demonstrated that the cultured AVICs express SM α-actin, vimentin and taurine transporter (TAUT), but not CD31, SM-myosin or desmin. We also established the osteoblastic differentiation model of the AVICs induced by pro-calcific medium (PCM) containing β-glycerophosphate disodium, dexamethasone and ascorbic acid in vitro. The results showed that taurine attenuated the PCM-induced osteoblastic differentiation of AVICs by decreasing the alkaline phosphate (ALP) activity/expression and the expression of the core binding factor α1 (Cbfα1) in a dose-dependent manner (reaching the maximum protective effect at 10 mM), and taurine (10 mM) inhibited the mineralization level of AVICs in the form of calcium content significantly. Furthermore, taurine activated the extracellular signal-regulated protein kinase (ERK) pathway via TAUT, and the inhibitor of ERK (PD98059) abolished the effect of taurine on both ALP activity/expression and Cbfα1 expression. These results suggested that taurine could inhibit osteoblastic differentiation of AVIC via the ERK pathway.

Keywords Taurine · Aortic valve interstitial cells · Extracellular signal-regulated protein kinase

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Introduction

Aortic valve calcification (AVC) characterized by the deposition of calcified plaques, thickening and rigidity of the leaflets is a kind of age-related lesion of the aortic valve with a prevalence of 25% among the senile population (>65 years) (Stewart et al. 1997). Severely developed AVC could lead to aortic stenosis or regurgitation, which may deteriorate the left heart function. AVC was once described as "degenerative and irreversible" (Sell and Scully 1965). Currently, the only effective therapy is aortic valve replacement, partly because the mechanisms of AVC are poorly understood. Recently, active progression was noticed in the pathogenesis of AVC including the osteoblastic differentiation of aortic valve interstitial cells (AVICs) and subsequent osteogenesis (Mohler et al. 2001; Rajamannan et al. 2003).

AVICs are different from the other cell types in the human body in that they function as both the fibroblasts (secreting collagen and shaping the extracellular matrix) and smooth muscle cells (contracting according to the tension of the aortic valves) (Taylor et al. 2003; Chester and Taylor 2007). Studies in vitro revealed that a subgroup of AVICs has the potential to differentiate into osteoblastic-like cells by expressing osteoblastic markers, including core binding factor α1 (Cbfα1/RUNX2), alkaline phosphatase (ALP/

AKP), osteocalcin (OC) and osteopontin (OPN), and forming calcific nodules induced by the pro-calcific medium (Chen et al. 2009; Osman et al. 2006). Additionally, some cytokines, bone morphogenetic proteins and medications could promote or suppress the osteoblastic differentiation of AVICs (Kaden et al. 2005; Clark-Greuel et al. 2007; Rabkin et al. 2009; Osman et al. 2006). These findings suggest that AVC might be (or partially be) an active process that could be prevented before it is too late.

Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in mammalian tissues with an intracellular concentration of 20–70 mmol/kg in heart and skeletal muscles (Huxtabe 1992; Chapman et al. 1993), and it is pumped into cells via the Na^+-dependent taurine transporter (TAUT). Although taurine does not form proteins in the body, it plays crucial roles in many physiological processes such as bile acid conjugation, detoxification, membrane stabilization and calcium flux. Deficiency of taurine will lead to or increase the risk of cardiovascular diseases, diabetes, and neurological and hepatic disorders (reviewed in "Taurine-monograph" 2001). A worldwide epidemiological study (Yamori et al. 2010) has found that populations with less taurine intake are prone to have higher body mass index, blood pressure, total cholesterol (T-Cho) and atherogenic index and higher incidence of cardiovascular diseases, which are all risk factors of AVC, suggesting taurine might be preventive against AVC. Our previous studies revealed that TAUT was expressed on the vascular smooth muscle cells (VSMCs) (Liao et al. 2007) and taurine could inhibit the osteoblastic differentiation of VSMCs induced by β-GP via the extracellular signal-regulated protein kinase (ERK) pathway (Liao et al. 2008). The results indicated that taurine might act against vascular calcification, while its effect on the AVC is still unknown. The aim of this study was to explore the effect of taurine on the osteoblastic differentiation of human AVICs and the underlying mechanisms.

Materials and methods

Isolation and culture of cells

Human aortic valves were collected from four patients of acute aortic dissection (Type A) when performing aortic valve replacement at the the Second Xiang-Ya Hospital, Central South University. All the patients had no previous history of heart valve disease with a mean age of 29.8 ± 8.5 years. All minute lesions of leaflets were excluded by pathological examinations. This study was approved by the ethics committee of the Second Xiang-Ya Hospital, and all patients signed informed consents.

AVICs were isolated as previously described (Meng et al. 2008) with some modifications. The distal 2/3 of the aortic valve leaflets were excised in the operating room and rinsed in PBS with penicillin G/streptomycin/amphotericin B (200 U/ml) within 2 h. The leaflets were digested in 2.5 mg/ml type II collagenase and 0.125% trypsin with 0.02% EDTA in 4.5 g/l glucose Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) for 30 min at 37°C to remove the endothelial cells by vortexing. The leaflets were cut into small pieces ($\sim 1 \times 1$ mm) and further digested with 0.8 mg/ml type II collagenase and 0.125% trypsin with 0.02% EDTA for 90 min at 37°C by vortexing for 10 s every 15 min. The softened leaflet fragments with free cells were spun at 500*g* for 2 min and the supernatant was aspirated. The precipitate was resuspended in full medium (4.5 g/l glucose DMEM with 10% fetal bovine serum (Hyclone), 200 U/ml penicillin G/streptomycin/amphotericin B), then plated onto a six-well plate and cultured in a cell culture incubator supplied with 5% CO_2. On reaching 80–90% confluence, the cells were sub-cultured in 75 cm^2 flasks, and passages 3–6 were used for these experiments. Taurine (Sigma), PD98059 (Sigma) and β-alanine (China National Medicine Corporation Ltd) used for intervention were all used as co-treatment in separate experiments.

Identification of cells by immunochemistry staining

To make sure that the cultured cells were not contaminated by other cells and expressed TAUT, the cultured cells were digested and plated onto a 48-well plate, washed with PBS and fixed in 4% paraformaldehyde for 5 min. Cells were blocked in non-immune goat serum and incubated with anti-human TAUT (Sigma), SM α-actin, vimentin, SM-myosin, desmin and CD31 (Santa Cruz) antibody for 1 h at room temperature. Cells were washed with PBS and incubated in the goat anti-mouse or anti-rabbit antibody conjugated with biotin for 1 h at room temperature. Cells were washed in PBS and incubated in streptavidin conjugate horseradish peroxidase. Cell were then washed with PBS and stained by the AEC method. Stained cells were visualized using a Nikon TE300 phase contrast microscope.

Osteoblastic differentiation of cells

To establish the calcification model of the AVICs, cultured cells at $\sim$90% confluence were placed into pro-calcific medium (PCM) containing 10 mM beta-glycerophosphate disodium (β-GP), 10 nM dexamethasone (DXM) and 50 μg/ml ascorbic acid (VitC). The first day of culture in the PCM was defined as day 0. On the 21st day of the experiment, the ALP expression was detected by 4-chloro-3-indolyl phosphate/nitroblue tetrazolium chloride (BCIP/NBT) method. The calcification of cells was detected on the 21st day by von Kossa staining.

Analysis of alkaline phosphatase activity

The cells treated for 21 days in six-well plates were washed three times with PBS and lysed in 1× cell lysis buffer (Cell Signaling Technology) with complete EDTA-free protease inhibitor (Roche) on ice for 5 min, then scraped out and centrifuged. The supernatant was assayed by spectrophotometric measurement of *p*-nitrophenol release at 520 nm wave length immediately after 15 min in a water bath at 37°C. ALP activity was normalized to total protein content of the cell layer by the bicinchoninic acid (BCA) method.

Western blotting

The treated cells were lysed in 1× cell lysis buffer (Cell Signaling Technology) with complete EDTA-free protease inhibitor (Roche). Protein content was homogenized using the BCA method. Denatured protein samples with 1× loading buffer were separated by 12% polyacrylamide gel electrophoresis followed by transfer to a pure nitrocellulose blotting membrane (Pall Corporation). Proteins of interest were then blotted using antihuman antibodies (Santa Cruz), horseradish peroxidase conjugate second antibody (Proteintech) and Western blot chemiluminescence detection (Millipore). The expression of target protein was normalized to β-actin.

Quantification of calcium content in calcified plaques

The cells treated for 21 days were decalcified with 0.6 N HCl for 24 h. The concentration of [Ca^{2+}] in the supernatant was measured by atomic absorption spectroscopy. The remaining cells were washed with PBS for three times and lysed in 1× cell lysis buffer with complete EDTA-free protease inhibitor. The total protein content was measured by the BCA method. The calcium content was normalized to total protein content.

Statistics

The data were expressed as mean ± standard error of at least triplicate experiments, and representative experiments are shown. The comparisons among values of more than two groups were performed by one-way ANOVA. *P* value of 0.05 or less was considered significant.

Results

Phenotypic identification and osteoblastic differentiation of cultured AVICs

Cell phenotype was confirmed by positive immunostaining for SM α-actin (>95%), vimentin (interstitial cell) (100%) and TAUT (100%), and negative for CD31 (endothelial cell), SM-myosin (smooth muscle cell) and desmin (muscle cell) (Fig. 1a–g). Cultured AVICs induced by PCM were positive for ALP staining and von Kossa staining on the 21st day of the intervention (Fig. 1h, i). Anderson et al. (2009) reported that PAT1 (SLC36A1) is another taurine transporter which is even more efficient than TAUT in transporting taurine into cells; however, the ideal pH value for PAT1 to work is 5.5, which is common in gut cavity but not cardiovascular system. Thus, we did not detect the existence or function of PAT1 in this study.

Taurine inhibits osteoblastic differentiation and mineralization of AVICs

To determine the effect of taurine on the osteoblastic differentiation of AVICs, we stimulated the cells by PCM with taurine of different concentrations, and the osteoblastic markers—ALP and Cbfα1—were detected. After 14 and 21 days of intervention, the ALP activity/expression in the PCM group was markedly higher than that of the blank control group, and taurine decreased the activity/expression of ALP dramatically in a dose-dependent manner (Fig. 2a–d). The effect of taurine started at 1 mM, became more obvious as the concentration rose and reached the maximum at 10 mM. Taurine of 20 mM was less protective than that of 10 mM. The ERK pathway inhibitor PD98059 (10 μM) blocked the inhibitory effect of taurine on the ALP activity/expression. Taurine or PD98059 (10 μM) alone had no influence on the ALP activity/expression. AVICs induced by PCM for 24 h expressed more Cbfα1 than the control group. Taurine suppressed the Cbfα1 expression and PD98059 (10 μM) abolished the suppression effect of taurine (10 mM) significantly (Fig. 3a, b). The expression of SM α-actin, vimentin and TAUT was not affected by PCM with/without taurine (10 mM) markedly (Fig. 4a–c). Taurine (10 mM) inhibited the mineralization level of AVICs in the form of calcium content significantly (Fig. 5a, b).

Taurine activated the ERK pathway in AVICs

Taurine (10 mM) activated the ERK pathway of AVICs after 5 min of incubation, and the activation effect was diminished by the addition of PD98059 and β-alanine (30 mM) which blocked the entrance of taurine into the cells through TAUT (Fig. 6a, b).

Discussion

Calcified plaques in AVC are formed in two ways—cell death-induced dystrophic calcification and osteoblastic differentiation-induced osteogenesis, and the former is the

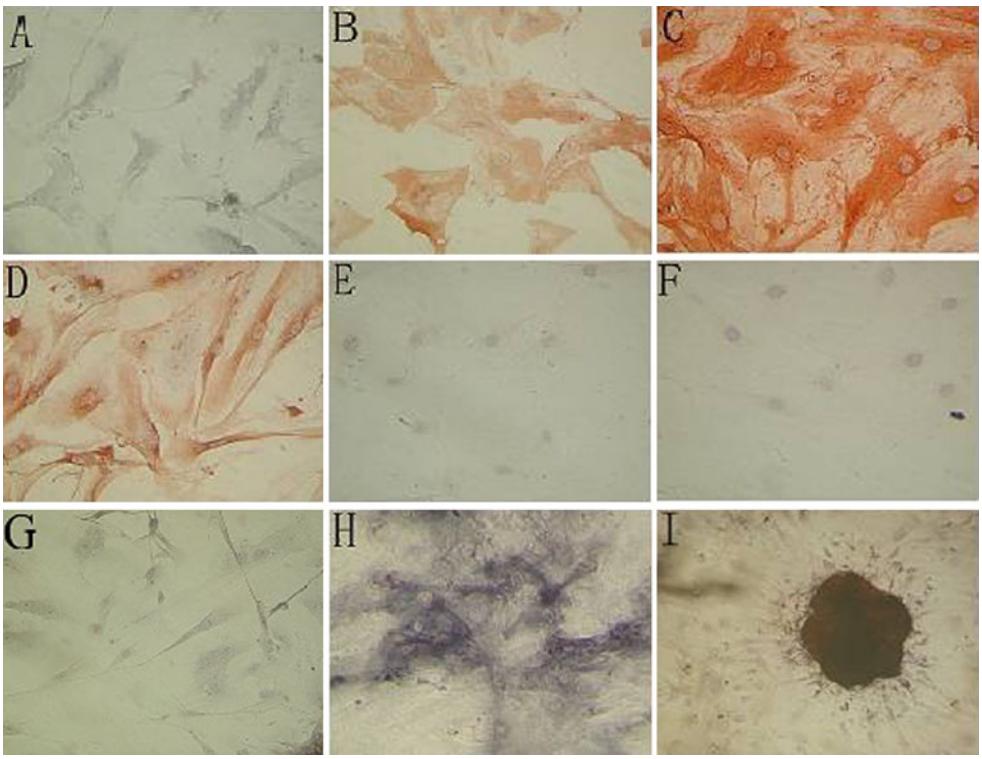

Fig. 1 Staining of cultured AVICs. **a** Negative control, **b** SM α-actin, **c** vimentin, **d** TAUT, **e** CD31, **f** SM-myosin, **g** desmin, **h** ALP staining, **i** von Kossa staining (**a**–**h** ×400, **i** ×100)

main type (Mohler et al. 2001). However, osteogenesis appears to be the advanced stage of calcification, in that osteoblastic markers turn up on the basis of adjacent dead AVICs (Mohler et al. 1999) and the formation of laminar bone is easier on the amorphous dystrophic calcification plaque, which might serve as a scaffold (Sage et al. 2010). ALP is frequently used as a downstream marker to evaluate osteoblastic differentiation, in that it plays a key role in the forming of the osteoid. ALP secreted in the extracellular matrix (ECM) directly cleaves the pyrophosphate, which inhibits calcium-phosphate crystal formation (Murshed et al. 2005). Mathieu et al. (2005) proved that ALP activity in AVICs was correlated with calcium content, and levamisole (an ALP inhibitor) inhibits β-GP induced calcification process. Another osteoblastic marker Cbfα1, expressed only in osteoblasts, is a key transcription factor which binds to the osteoblast-specific cis-acting element OSE2. The silencing of Cbfα1 expression markedly downregulated the expression of type I collagen, OC and OPN, which are all markers of osteoblasts (Ducy et al. 1997). Furthermore, in both human bodies and animal models of AVC, calcified aortic leaflets were detected with high expression of Cbfα1 (Mohler et al. 2001; Rajamannan et al. 2002). Whether the expression of ALP is regulated by Cbfα1 or not is unknown. SM α-actin is the dominant/specific phenotype in AVICs (Taylor et al. 2003) and the expression rate is up to 95% in this study. It is reported that transforming growth factor-β can promote the expression of SM α-actin in a dose-dependent manner and SM α-actin might be the only contractile component in AVICs (Walker et al. 2004). However, there is little change in the expression of SM α-actin in PCM (±taurine) compared with control under our observation, which suggests that neither PCM nor taurine might have influence on the contractility of AVICs. Vimentin is a kind of intermediate filament protein and the major cytoskeletal component of mesenchymal cells, which is commonly used as a biomarker for interstitial cells in the laboratory. We found that the expression of vimentin is not changed significantly in PCM (±taurine). In this study, we confirmed that cultured human AVICs express ALP and Cbfα1 and form calcified nodules induced by PCM. Taurine inhibits the expression of ALP and Cbfα1 and reduces calcium content in vitro.

Taurine, as a metabolite of methionine and cysteine, is the most abundant amino acid in animal tissues (millimolar concentrations) (Huxtabe 1992; Chapman et al. 1993). It had no cytotoxicity against human AVICs at concentrations between 1 and 20 mM in our study. In the cardiovascular system, taurine has multiple effects including lowering blood pressure, preventing coronary artery disease, alleviating physical signs and symptoms of congestive heart failure, and even reversing ECG abnormalities (Birdsall 1998). Recently, Li et al. (2004) demonstrated that taurine suppressed the calcification in VSMCs induced

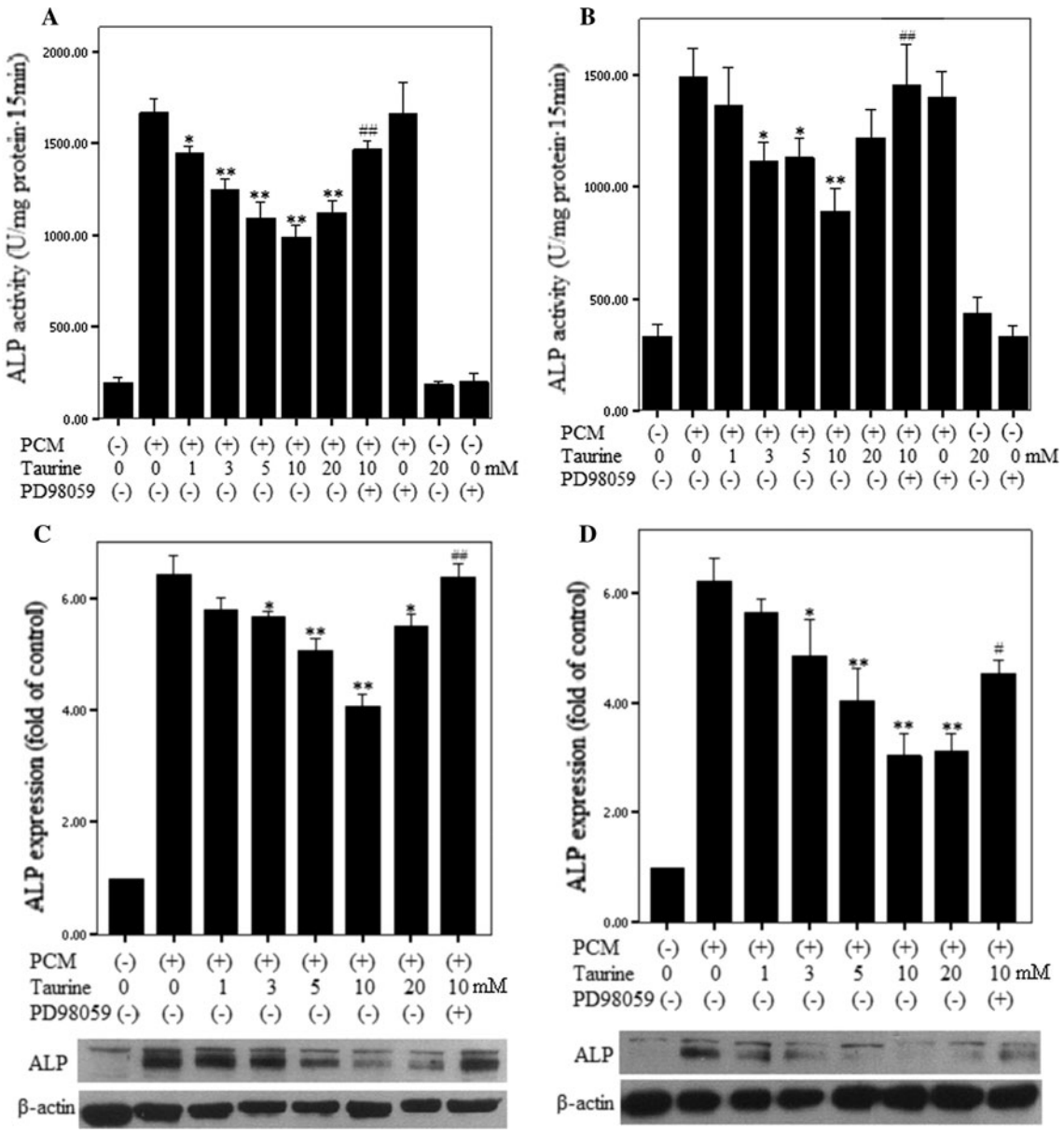

Fig. 2 The effect of taurine on the ALP activity/expression. Human AVICs were induced by PCM with taurine of different concentrations and PD98059 for 14 days (**a**, **c**) and 21 days (**b**, **d**), and the cell lysates were subjected to ALP activity assay (**a**, **b**) and Western blotting (**c**, **d**). (*$P < 0.05$ vs. PCM group, **$P < 0.01$ vs. PCM group, $^{\#}P < 0.05$ vs. PCM plus 10 mM taurine group, $^{\#\#}P < 0.01$ vs. PCM plus 10 mM taurine group)

by β-GP and our team showed that taurine could inhibit osteoblastic differentiation of VSMCs via ERK activation (Liao et al. 2008). However, Park et al. (2001) found that taurine (10 mM) enhanced the synthesis of ALP and collagen in osteoblast-like UMR-106 cells via ERK. In this study, we confirmed that taurine inhibits the expression of ALP and Cbfα1 of AVICs in a dose-dependent manner, but taurine of 20 mM was less protective than 10 mM. Considering these previous findings and the present results, we deduced that taurine played different roles in calcification in different tissues, and the effect of taurine might be related to the concentration as well. The effect of ERK in ectopic calcification is controversial at present. Ding et al. (2006) found that PD98059 blocked fibronectin-induced osteoblastic differentiation of VSMCs, and Gu and Masters (2009) proved that the content of p-ERK was elevated in calcifying AVICs cultured on polystyrene as well as fibrin-coated plates, and PD98059 inhibited the calcification degree. On the contrary, Radcliff et al. (2005) demonstrated that insulin-like growth factor-I (IGF-I) elevated the expression of p-ERK in VSMCs, and PD98059 suppressed the protective effect of IGF-I on the calcification of

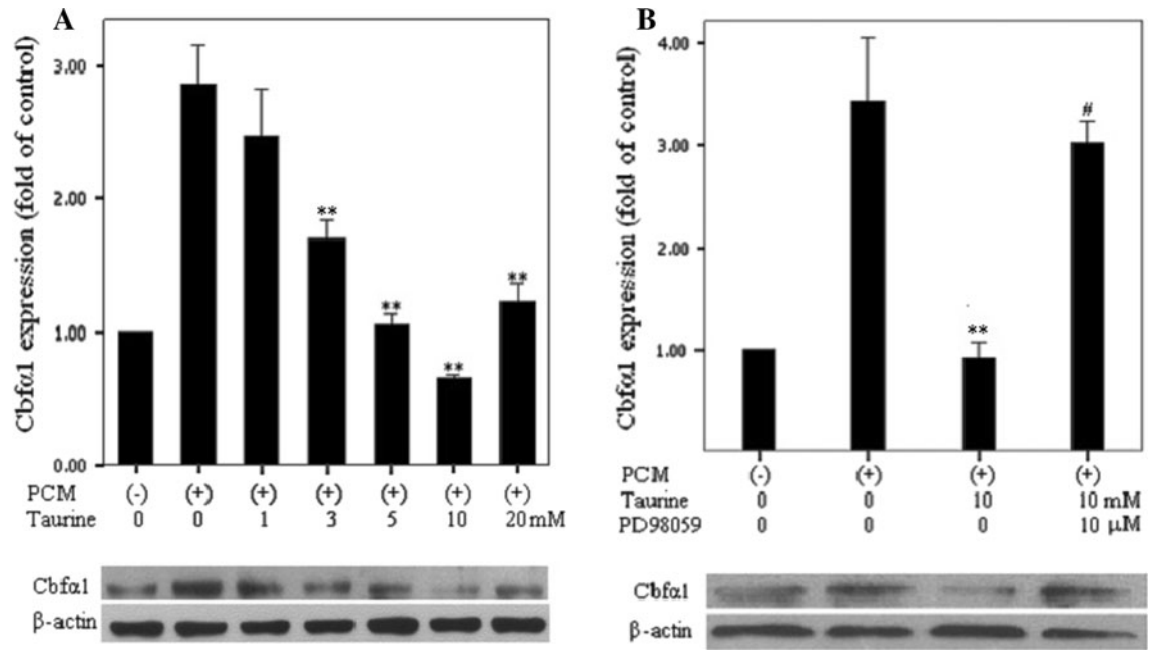

Fig. 3 The effect of taurine on the expression of Cbfα1 in AVICs induced by PCM. **a** Cultured AVICs were treated with PCM with taurine of different concentrations for 24 h. Western blotting showed that taurine suppressed the expression of Cbfα1 in a dose-dependant manner. **b** Addition of PD98059 (10 μM) blocked the effect of taurine (10 mM) on Cbfα1 expression ($^{**}P < 0.01$ vs. PCM group, $^{\#}P < 0.01$ vs. PCM plus 10 mM taurine group)

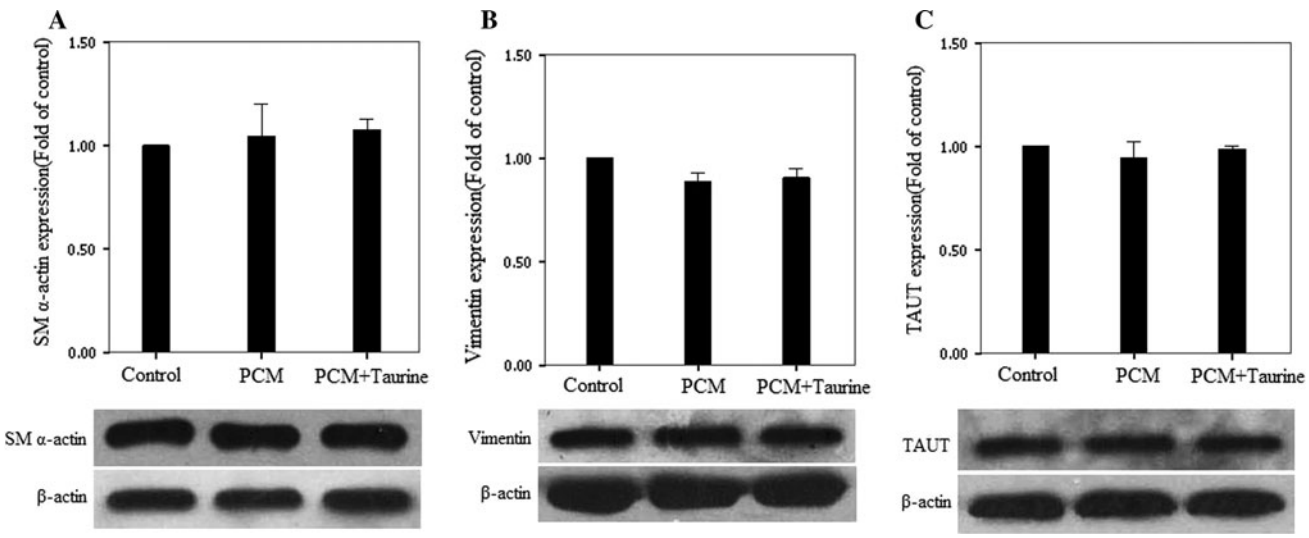

Fig. 4 The expression of SM α-actin, vimentin and TAUT on the 21st day of intervention. Western blotting showed that neither PCM nor PCM plus taurine (10 mM) could affect the expression of SM α-actin (**a**), vimentin (**b**), and TAUT (**c**) significantly

VSMCs induced by β-GP. In addition, our previous (Liao et al. 2008) and present work suggested that taurine activated the ERK pathway, and the blockade of ERK diminished the protective effect of taurine in VSMCs and AVICs. Therefore, more studies are needed to elucidate the role of ERK pathway in ectopic calcification.

TAUT (SLC6A6), which has a 12-loop transmembrane domain, is expressed broadly in mammalian tissues and pumps taurine into cells with Na^+ and Cl^-. The expression of TAUT was not changed significantly during the intervention of PCM (±taurine) in vitro in this study. However, it is still unknown whether there is change in the expression of TAUT between healthy and calcified aortic valve leaflets in vivo, which needs further investigation. β-Alanine, as a competitive substrate, is known as the inhibitor of TAUT, because taurine and β-alanine are both β-amino acids sharing similar chemical structure (Takeuchi et al. 2000). In this study, we

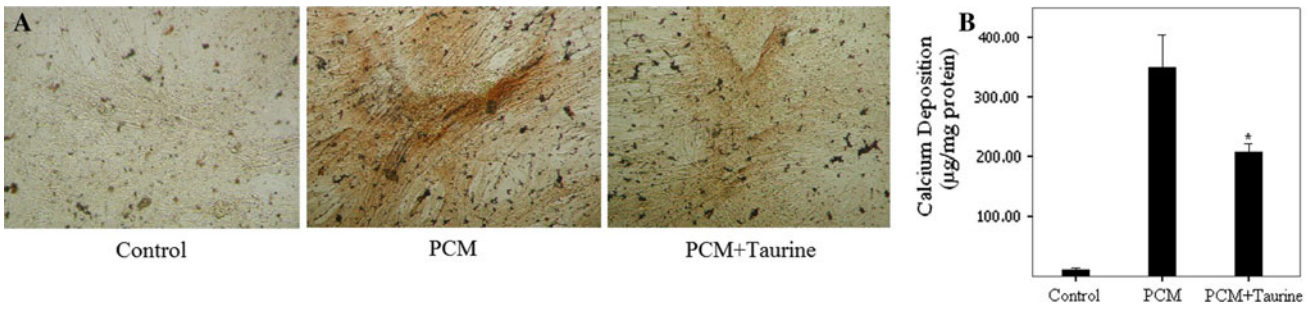

Fig. 5 Taurine (10 mM) suppressed the mineralization level of AVICs on the 21st day of intervention. **a** von Kossa staining of the calcified plaques (×100). **b** Calcium content was reduced by taurine significantly (*$P < 0.05$ vs. PCM group)

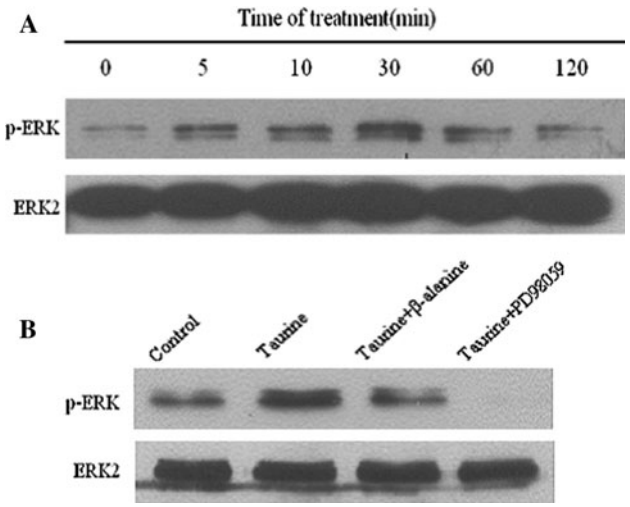

Fig. 6 The activation effect of taurine on the ERK pathway. AVICs were incubated in serum-free medium for 5 h before the intervention. **a** Western blotting shows the expression of p-ERK induced by taurine (10 mM) for the indicated time. **b** The activation of ERK by taurine (10 mM) was blocked by alanine (30 mM) and PD98059 (10 μM) in 20 min

demonstrated that β-alanine blocked the activation effect of taurine on the phosphorylation of ERK.

In conclusion, we proved that human AVICs express TAUT and have the capacity of expressing osteoblastic markers (ALP and Cbfα1) and forming calcified nodules induced by PCM. We found that taurine inhibited the expression of ALP and Cbfα1 in a dose-dependent manner and suppressed the mineralization level of AVICs, and the ERK pathway takes part in the protective effect of taurine. These findings suggest that human AVICs play a major role in AVC which could be induced by potential mediators and attenuated by taurine.

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References

Anderson CM, Howard A, Walters JR, Ganapathy V, Thwaites DT (2009) Taurine uptake across the human intestinal brush-border membrane is via two transporters: H^+-coupled PAT1 (SLC36A1) and Na^+- and Cl^--dependent TauT (SLC6A6). J Physiol 587(Pt 4):731–744

Birdsall TC (1998) Therapeutic applications of taurine. Altern Med Rev 3(2):128–136

Chapman RA, Suleiman MS, Earm YE (1993) Taurine and the heart. Cardiovasc Res 27(3):358–363

Chen JH, Yip CY, Sone ED, Simmons CA (2009) Identification and characterization of aortic valve mesenchymal progenitor cells with robust osteogenic calcification potential. Am J Pathol 174(3):1109–1119

Chester AH, Taylor PM (2007) Molecular and functional characteristics of heart-valve interstitial cells. Philos Trans R Soc Lond B Biol Sci 362(1484):1437–1443

Clark-Greuel JN, Connolly JM, Sorichillo E, Narula NR, Rapoport HS, Mohler ER 3rd, Gorman JH 3rd, Gorman RC, Levy RJ (2007) Transforming growth factor-β1 mechanisms in aortic valve calcification: increased alkaline phosphatase and related events. Ann Thorac Surg 83(3):946–953

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(5):1343–1352

Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G (1997) Osf2/Cbfα1: a transcriptional activator of osteoblast differentiation. Cell 89(5):747–754

Gu X, Masters KS (2009) Role of the MAPK/ERK pathway in valvular interstitial cell calcification. Am J Physiol Heart Circ Physiol 296(6):H1748–H1757

Huxtabe RJ (1992) Physiological actions of taurine. Physiol Rev 72(1):101–163

Kaden JJ, Kiliç R, Sarikoç A, Hagl S, Lang S, Hoffmann U, Brueckmann M, Borggrefe M (2005) Tumor necrosis factor alpha promotes an osteoblast-like phenotype in human aortic valve myofibroblasts: a potential regulatory mechanism of valvular calcification. Int J Mol Med 16(5):869–872

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(3):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(4):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(4):525–530

Mathieu P, Voisine P, Pépin A, Shetty R, Savard N, Dagenais F (2005) Calcification of human valve interstitial cells is dependent on alkaline phosphatase activity. J Heart Valve Dis 14(3):353–357

Meng X, Ao L, Song Y, Babu A, Yang X, Wang M, Weyant MJ, Dinarello CA, Cleveland JC Jr, Fullerton DA (2008) Expression of functional toll-like receptors 2 and 4 in human aortic valve interstitial cells: potential roles in aortic valve inflammation and stenosis. Am J Physiol Cell Physiol 294(1):C29–C35

Mohler ER 3rd, Chawla MK, Chang AW, Vyavahare N, Levy RJ, Graham L, Gannon FH (1999) Identification and characterization of calcifying valve cells from human and canine aortic valves. J Heart Valve Dis 8(3):254–260

Mohler ER 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS (2001) Bone formation and inflammation in cardiac valves. Circulation 103(11):1522–1528

Murshed M, Harmey D, Millán JL, McKee MD, Karsenty G (2005) Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone. Genes Dev 19(9):1093–1104

No authors listed (2001) Taurine-monograph. Altern Med Rev 6(1):78–82

Osman L, Yacoub MH, Latif N, Amrani M, Chester AH (2006) Role of human valve interstitial cells in valve calcification and their response to atorvastatin. Circulation 114(Suppl 1):I547–I552

Park S, Kim H, Kim SJ (2001) Stimulation of ERK2 by taurine with enhanced alkaline phosphatase activity and collagen synthesis in osteoblast-like UMR-106 cells. Biochem Pharmacol 62(8): 1107–1111

Rabkin SW, Lodhia P, Luong MW (2009) P38 MAP kinase in valve interstitial cells is activated by angiotensin II or nitric oxide/peroxynitrite, but reduced by toll-like receptor-2 stimulation. J Heart Valve Dis 18(6):653–661

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(4):398–400

Rajamannan NM, Subramaniam M, Springett M, Sebo TC, Niekrasz M, McConnell JP, Singh RJ, Stone NJ, Bonow RO, Spelsberg TC (2002) Atorvastatin inhibits hypercholesterolemia-induced cellular proliferation and bone matrix production in the rabbit aortic valve. Circulation 105(22):2660–2665

Rajamannan NM, Subramaniam M, Rickard D, Stock SR, Donovan J, Springett M, Orszulak T, Fullerton DA, Tajik AJ, Bonow RO, Spelsberg T (2003) Human aortic valve calcification is associated with an osteoblast phenotype. Circulation 107(17):2181–2184

Sage AP, Tintut Y, Demer LL (2010) Regulatory mechanisms in vascular calcification. Nat Rev Cardiol 7(9):528–536

Sell S, Scully RE (1965) Aging changes in the aortic and mitral valves: histologic and histochemical studies, with observations on the pathogenesis of calcific aortic stenosis and calcification of the mitral annulus. Am J Pathol 46:345–365

Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, Smith VE, Kitzman DW, Otto CM (1997) Clinical factors associated with calcific aortic valve disease. Cardiovascular health study. J Am Coll Cardiol 29:630–634

Takeuchi K, Toyohara H, Sakaguchi M (2000) A hyperosmotic stress-induced mRNA of carp cell encodes Na^{+}- and Cl^{-}-dependent high affinity taurine transporter. Biochim Biophys Acta 1464(2):219–230

Taylor PM, Batten P, Brand NJ, Thomas PS, Yacoub MH (2003) The cardiac valve interstitial cell. Int J Biochem Cell Biol 35(2): 113–118

Walker GA, Masters KS, Shah DN, Anseth KS, Leinwand LA (2004) Valvular myofibroblast activation by transforming growth factor-beta: implications for pathological extracellular matrix remodeling in heart valve disease. Circ Res 95(3):253–260

Yamori Y, Taguchi T, Hamada A, Kunimasa K, Mori H, Mori M (2010) Taurine in health and disease: consistent evidence from experimental and epidemiological studies. J Biomed Sci 17(Suppl 1):s6