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ORIGINAL ARTICLE

Chondrocytic cells express the taurine transporter on their plasma membrane and regulate its expression under anisotonic conditions

Hannu M. Karjalainen · Chengjuan Qu · Stina S. Leskelä · Kirsi Rilla · Mikko J. Lammi

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Abstract Taurine is a small organic osmolyte which participates in cell volume regulation. Chondrocytes have been shown to accumulate and release taurine; in bone, taurine participates in bone metabolism. However, its role in skeletal cells is poorly understood, especially in chondrocytes. This study investigated the regulation of taurine transporter in chondrocytic cells. We examined the transcriptional regulation of the taurine transporter under anisotonia by reporter gene and real-time RT-PCR assays. The effect of providing supplementary taurine on cell viability was evaluated with the lactate dehydrogenase release assay. The localization of the taurine transporter in human chondrosarcoma cells was studied by overexpressing a taurine transporter-enhanced green fluorescent protein. We observed that the transcription of the taurine transporter gene was up-regulated in hypertonic conditions. Hyperosmolarity-related cell death could be partly abolished by taurine supplementation in the medium. As expected, the fluorescently labeled taurine transporter localized at the plasma membrane. In polarized epithelial MDCK cells, the strongest fluorescence signal was located in the lateral cell membrane area. We also observed that the taurine transporter gene was expressed in several human tissues and malignant cell lines. This is the first study to present information on the transcriptional regulation of taurine transporter gene and the localization of the taurine transporter protein in chondrocytic cells.

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Introduction

Taurine (2-aminoethanesulfonic acid) is a small organic compound which can be present at high intracellular concentrations, particularly in retinal, brain, heart, muscle and kidney cells (Huxtable 1992). It has many physiological and pathophysiological functions (Yamori et al. 2010). During a hypertonic challenge, cells actively accumulate small biochemically inert molecules, osmolytes, into the cytosol, and release them to the interstitial matrix under hypotonic conditions. This is a common mechanism through which cells resist volume changes, such as those occurring during osmotic stress (Turunen et al. 2012). Taurine is a ubiquitous osmolyte that is involved in cell volume regulation (Hoffmann et al. 2009; Lang 2007), and the taurine transport and release mechanism have been one of the most widely studied cellular volume regulation systems (Lambert 2004). In addition, it plays an important role in the formation of bile salts (Kevresan et al. 2006), as well as in the maintenance of intracellular calcium homeostasis (Conte Camerino et al. 2004), suppression of reactive oxygen species generation (Ito et al. 2009), prevention of cell death (Ripps and Shen 2012; Yancey 2005), and it may even act as an microbicidal agent when it is in the forms of taurine chloramine and bromamine (Marcinkiewicz and Kontny 2012).

The published literature is sparse on the role of taurine in skeletal cell types, such as chondrocytes, osteoblasts, osteoclasts and mesenchymal stem cells. Cartilage tissue is a special structure composed of a collagen network and proteoglycans, which create a unique osmotic environment for the chondrocytes (Urban 1994). The fixed

negative charges of proteoglycans which are trapped within the tissue by the dense three-dimensional collagen network attract cations and water producing a high swelling pressure in the cartilage tissue. The osmotic pressure of plasma (~300 mOsm/l) is generally considered to be isotonic. However, the osmolarity in articular cartilage varies between 350 and 450 mOsm/l, depending on the local proteoglycan concentration (Hall and Bush 2001). This range can be expected to be even higher when compressive loading squeezes water out from articular cartilage. In osteoarthritic cartilage, the osmotic pressure may fall below this range due to a decrease in the proteoglycan content. Thus, chondrocytes have to be able to adapt to the "isotonic" conditions of roughly 400 mOsm/l and, more importantly, be able to withstand rather abrupt and major changes in their osmotic environment. Thus, it can be assumed that taurine plays an important role in the regulation of the cell volume in chondrocytes; in fact, the primary chondrocytes have been shown to utilize this mechanism to regulate their cellular volume in response to an osmotic challenge (Hall 1995; Hall and Bush 2001). However, detailed clarifications on the transcriptional regulation and protein localization of taurine transporter, the integral part of this system, have not been conducted in chondrocytes.

In osteoblasts, taurine has been reported to enhance alkaline phosphatase activity and osteocalcin expression (Yuan et al. 2006), to increase osteoblast proliferation (Jeon et al. 2007), and to inhibit osteoblast apoptosis (Zhang et al. 2011). On the other hand, taurine may inhibit osteoclastogenesis (Koide et al. 1999), a process which has been shown to be taurine transporter dependent (Yuan et al. 2010). In mesenchymal stem cells, taurine was recently reported to promote osteogenesis via Erk pathway (Zhou et al. 2014).

In this study, we characterized the transcription of the human taurine transporter in chondrocytes and chondrosarcoma cells during exposure to anisotonic conditions. We also examined the distribution of taurine transporter mRNA in several human tissues and malignant cancer cell lines. In addition, for the first time, we have determined the cellular localization of human taurine transporter protein in chondrosarcoma cells using a taurine transporter-enhanced green fluorescent protein fusion protein. The effects of taurine on chondrogenic and osteogenic differentiation markers have also been investigated.

Materials and methods

Cell cultures

Human chondrosarcoma cells (HCS-2/8) (Takigawa et al. 1989) were expanded in monolayer cultures on poly-D-lysine-coated dishes (Nunc, Roskilde, Denmark and Greiner, Frickenhausen, Germany). The cells were grown in α-modified Eagle's medium (α-MEM, HyClone, Logan, OH, USA) supplemented with 10 % fetal bovine serum (FBS, PAA Laboratories, Linz, Austria), 2 mM L-glutamine (Lonza, Verviers, Belgium), 100 U/ml of penicillin (Lonza), and 100 µg/ml of streptomycin (Lonza) in a regular cell culture incubator in a 37 °C at 5 % CO_2/95 % air atmosphere with 100 % relative humidity.

Primary bovine chondrocytes were isolated from 1- to 2-year-old animals (Qu et al. 2012). The isolated chondrocytes (1 million cells) were seeded on 35 mm culture plates in chondrocyte culture medium (Qu et al. 2012). The medium was changed three times a week until they reached 90–95 % confluency.

MG-63 osteoblasts were grown in α-MEM supplemented with 10 % FBS, 20 mM HEPES (Sigma), 100 units/ml penicillin, and 100 µg/ml streptomycin. All cells were maintained in a humidified, 95 % air, 5 % CO_2 atmosphere at 37 °C. The medium was changed three times a week.

Human mesenchymal stem cells were isolated from bone marrow materials (permission approved by North-Savo Health Care District Ethical Committee, license no. 62/2010). Bone marrow specimens from three donors were examined separately. The cells adhering to the plates were expanded until passage 2 as described previously and stored frozen until needed for further experiments (Kaitainen et al. 2013).

Northern blot analysis

Human 12-Lane Multiple Tissue Northern (MTN)™ Blot and Human Cancer Cell Line MTN® Blot II (Clontech Laboratories, Palo Alto, CA, USA) membranes were used to indicate cell- and tissue-specific expression of taurine transporter mRNA. A cDNA fragment of human taurine transporter mRNA (GenBank access number NM_003043.3, base pairs 6,076–6,230) was generated by reverse transcription-PCR and cloned into pCR®2.1-TOPO® plasmid (Life Technologies, Invitrogen, Paisley, UK). The plasmid was used to label a probe for Northern blot analysis. A plasmid containing the DNA sequence of glyceraldehyde-3-phosphate dehydrogenase (Fort et al. 1985) was used to prepare a probe for normalization. The probes were labeled with [α-^{32}P]-dCTP (Amersham Biosciences Little Chalfont, UK), using random primed labeling (High Prime, Roche Applied Science, Mannheim, Germany). Hybridization was carried out in Ultrahyb hybridization solution (Ambion, Austin, TX, USA) according to the manufacturer's instructions, and the detection was performed using the Storm™ Phosphorimager (Molecular Dynamics, Sunnyvale, CA, USA).

Taurine transporter expression in hypo- and hypertonic cell cultures

Subconfluent HCS-2/8 cells and bovine primary chondrocytes were exposed to anisotonic conditions by adding the culture medium described above, adjusted either to hypotonic osmolality (200 mOsm/l) by adding distilled water or made hypertonic (450 mOsm/l) by adding NaCl. The isotonic (300 mOsm/l) medium was supplemented with the corresponding volume of PBS. Cells were grown in anisotonic conditions for 0, 4, 8, 24, 48 or 96 h. Total RNA was extracted with Tri Reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instruction at the end of cell culture. The experiment was repeated three times.

Quantitative real-time RT-PCR (qRT-PCR)

The concentration of total RNA was determined by spectrophotometric measurement at 260 nm (Nanodrop, Wilmington, DE, USA). cDNA was generated with Verso cDNA Synthesis kit (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's recommendations using a MJ Research PTC-200 device (Waltham, MA, USA).

The 15 µl of RT-PCR reaction contained 4 µl (4 ng) of cDNA, 11 µl of Maxima SYBR Green/ROX qPCR Master Mix (Thermo), and 100 nM [human acidic ribosomal protein large P0 (RPLP0), taurine transporter, procollagen α_1(I), procollagen α_2(I), and Cbfa1/RUNX2] of forward and reverse primers. The quantitative real-time RT-PCR analyses of these genes were performed using Mx3000P™ Real-Time PCR System (Stratagene, La Jolla, CA, USA).

Taurine transporter promoter activity in hypo- and hypertonic cell cultures

Dual-luciferase® reporter gene assay (Promega, Madison, WI, USA) was performed as previously described (Qu et al. 2009), using taurine transporter firefly luciferase reporter plasmid, which contains −124 to +46 region of rat taurine transporter promoter (Han et al. 2000; Ito et al. 2004), and Renilla luciferase plasmid at 10:1 ratio. We also used a taurine transporter reporter plasmid, which has a mutated tonicity-responsive element (TonE) (Ito et al. 2004) to verify that the induction of the reporter gene by hypertonicity was being regulated by this region. Renilla luciferase activity was used to normalize the transfection efficiency. The transfection efficiency was previously shown to be 27 % (Qu et al. 2009). The experiment was repeated three times.

Taurine transporter promoter activity in dynamically stretched HCS-2/8 cells

Osmotic challenges are known to evoke changes in chondrocyte volumes, which create stretching forces in the cell membrane (Turunen et al. 2012). Therefore, we tested whether cellular stretching of HCS-2/8 cells would affect taurine transporter promoter activity. Two to four days before the exposure to stretching, chondrosarcoma cells were plated on dishes molded of silicone rubber (Elastosil 601-Me, Wacker Chemie, Munich, Germany). Prior to plating, the dishes were filled with 10 µg/ml poly-D-lysine (Sigma) solution for 6 h to increase adherence of the cells onto the dishes. Subconfluent cultures of HCS-2/8 cells were transfected with the taurine transporter and Renilla luciferase reporter plasmids, and on the next day they were subjected to 8 % cyclic stretching with High Strain Cell Stretching Apparatus (University of Ulm, Germany) at 1 Hz frequency for 6 and 24 h. The detailed specifications of this apparatus have been published previously (Neidlinger-Wilke et al. 1994). The samples were collected 24 h after the beginning of the stretching. Control cells were cultured similarly on silicone rubber dishes, excluding the stretching exposure. The experiment was repeated four times. Samples for qRT-PCR assays were also collected after 1, 3, 6, 12 and 24 h of the onset of the cellular stretching ($n = 3$).

Effect of taurine on chondrocyte and HCS-2/8 cell viability under osmotic challenge

Taurine has been reported to prevent hypertonia-induced cell death, for instance, in HepG2 cells (Ito et al. 2004). Since chondrocytes normally exist under hypertonic conditions, we tested whether taurine could protect primary chondrocytes or HCS-2/8 cells during an osmotic challenge. The cells were exposed to 200, 300 and 600 mOsm/l media for 6 h, and then cytotoxicity was assayed using LDH-Cytotoxicity Assay Kit II (Biovision, Milpitas, CA, USA). In parallel, taurine was included at 20 mM concentration to study its effect on the cell cultures. This experiment was conducted three times.

Confocal microscopy of transfected taurine transporter fusion protein

The taurine transporter is known to be a cell membrane protein, but as far as we are aware its precise location in living cells has not been demonstrated previously. In this study, we constructed a plasmid encoding an enhanced green fluorescent protein fused with the taurine transporter coding sequence. The coding sequence of human taurine transporter mRNA (1,860 bp) was amplified with RT-PCR using

Marathon™ cDNA Amplification Kit (Clontech Laboratories) using primer design to remove the stop codon. PCR product was then cloned into pEGFP-N1 vector to the same reading frame as the enhanced green fluorescent protein (Clontech Laboratories). The plasmid clone containing the correctly oriented taurine transporter insert was identified by DNA sequencing. The taurine transporter-pEGFP-N1 construct was transfected into HCS-2/8 cells using Exgen transfection reagent (Fermentas, St. Leon-Rot, Germany). At 24 h after transfection, the fusion protein was visualized in an Ultraview™ confocal scanner (Perkin Elmer Life Sciences, Wallac-LSR, Oxford, UK) on a Nikon Eclipse TE300 microscope and a 100 × oil immersion objective (Nikon Plan Fluor, NA 1,3). A three-dimensional model of the fusion protein localization was constructed by processing micrograph stacks with Vaytek Deconvolution 7.0 and VoxBlast 3.1 programs (VayTek, Fairfield, IA, USA). The scale was assessed using FocalCheck™ 6 µm fluorescent microspheres (Molecular Probes, Eugene, OR, USA) mounted on a slide.

Chondrocytes, osteoblasts and mesenchymal stem cells are not polarized cells. Since taurine transporter is strongly expressed in polarized kidney tubule epithelial cells, we wanted to investigate whether plasma membrane localization would be different in polarized cells. Madin-Darby canine kidney (MDCK) cells were transfected with taurine transporter-pEGFP-N1 plasmid with Exgen transfection reagent. Stably transfected cells were enriched by supplementing the medium after transfection with 800 µg/ml of G418. An insert-free pEGFP-N1 plasmid was transfected separately as a control. The monolayers were cultured on chambered coverglasses (Ibidi GmbH, Martinsried, Germany) and imaged with a confocal microscope (Carl Zeiss Microimaging GmbH, Jena, Germany). To obtain three-dimensional cultures with epithelial structures, stably transfected MDCK cells were mixed with basement membrane extract gel Matrigel™ (BD Biosciences, Bedford, MA) to create a suspension of 2×10^5 cells/ml. Aliquots of the suspension were portioned onto chambered coverglasses. After gelling at 37 °C, culture medium was added into the wells, and the cells were cultured at 37 °C. Fresh medium was changed every second day. After incubation for 7 days, the living cultures were inspected under a confocal microscope to visualize the taurine transporter.

Alkaline phosphatase activity of MG-63 osteoblasts after taurine treatment

MG-63 osteoblasts were cultured to 90–95 % confluency in a medium, which consisted of α-MEM supplemented with 10 % FBS, 100 IU/ml penicillin, and 100 µg/ml streptomycin. The cells were washed with serum-free medium, and new medium with or without 10 mM taurine being present for 24 h. After washing with PBS, the cells were lysed on ice into 1 ml of 10 mM Tris–HCl buffer containing 0.1 % Triton X-100 and then centrifuged. The lysates were homogenized with ultrasound, and alkaline phosphatase activity was determined by a spectrophotometric assay which measures the release of p-nitrophenol from its phosphate substrate at 37 °C. The activity was normalized to the protein content, which was measured using the Bradford assay (Bradford 1976). The experiment was repeated three times.

Mesenchymal stem cell chondrogenesis and osteogenesis assays in the presence of taurine

Bone marrow-derived mesenchymal stem cells from three donors were used in the chondrogenesis and osteogenesis assays in the absence or presence of 20 mM taurine supplement. The assays were performed as previously described (Kaitainen et al. 2013), except for using 50 ng of TGF-β_3 in the chondrogenic differentiation experiment.

Chondrogenesis was evaluated by histological analyses. At the end of 2 and 4 weeks of chondrogenic differentiation, the pellets were fixed in 4 % paraformaldehyde and 3-µm-thick sections were stained with toluidine blue for proteoglycans or immunostained for type II collagen (Qu et al. 2012). Immunohistochemical staining was performed with anti-type II collagen mouse monoclonal antibody E8 (Holmdahl et al. 1986).

Osteogenesis was evaluated by assaying alkaline phosphatase activity after 1, 2, 3 and 4 weeks from the beginning of the differentiation in the same way as conducted with the MG-63 osteoblasts. The cells were also fixed and stained for alkaline phosphatase (Qu et al. 2013). The gene expressions of procollagen α_1(I), procollagen α_2(I) and Cbfa1/RUNX2 were also analyzed by quantitative RT-PCR from samples collected weekly after the beginning of the differentiation.

Statistical analyses

Statistical tests were performed using the SPSS Statistic 16.0 software (SPSS Inc., Chicago, IL, USA). The results are given as means [95 % confidence intervals (CIs)]. Student's paired *t* test was used to check for statistical significance. Statistical significance was set at p less than 0.05.

Results

Taurine transporter is widely expressed in several human tissues and cancer cell lines

Commercially available Northern blots made it possible to estimate the expression of taurine transporter mRNAs

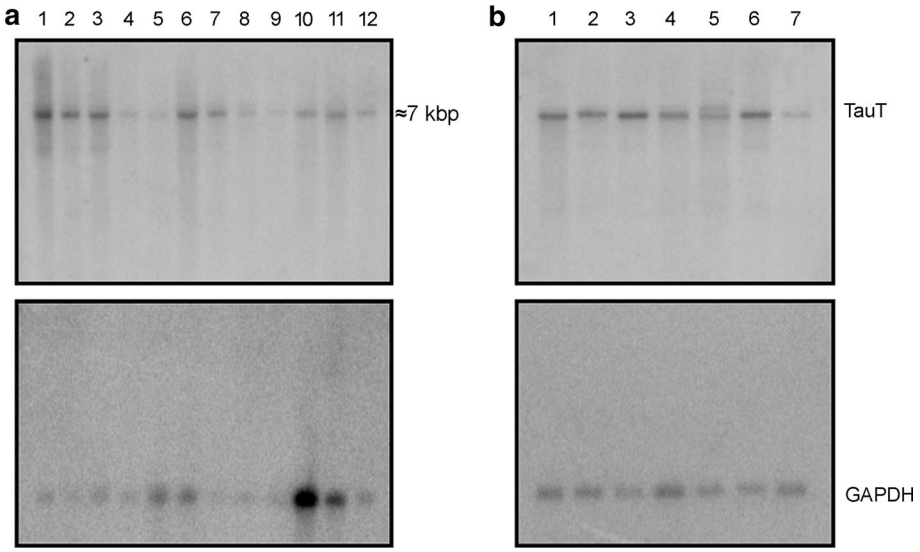

Fig. 1 Northern blot analysis of taurine transporter mRNA in various tissues (**a**) and carcinoma cell lines (**b**). The *lanes* in **a** represent the following sources: *1* leukocytes, *2* lung, *3* placenta, *4* small intestine, *5* liver, *6* kidney, *7* spleen, *8* thymus, *9* colon, *10* muscle, *11* heart and *12* brain. The *lanes* in **b** represent samples from *1* melanoma G361, *2* lung carcinoma A549, *3* colon carcinoma SW480, *4* lymphoblastic leukemia MOLT-4, *5* chronic myelogenous leukemia K-562, *6* HeLa cell S3 and *7* promyelocytic leukemia HL-60 cells. Glyceraldehyde-3-phosphate dehydrogenase was used for normalization

in several human tissues and malignant cancer cell lines. Approximately, 7 kb taurine transporter mRNA was abundantly expressed in leukocytes, lung, placenta, kidney, spleen, striated muscle and heart. Less extensive expressions were detected in small intestine, liver, thymus, colon and brain (Fig. 1a). The expression level of glyceraldehyde-3-phosphate dehydrogenase was not optimally equivalent in the multi-tissue membrane, but the hybridization is still indicative of the general levels. Only one cancer cell line (promyelocytic leukemia cells) out of the seven studied did not display abundant expression of the taurine transporter transcript (Fig. 1b).

The gene expression of the taurine transporter is regulated by an osmotic environment

The effect of an osmotic challenge on taurine transporter gene activity was evaluated with the dual-luciferase reporter gene assay. We used plasmids containing both the wild-type promoter region of rat taurine transporter and a plasmid with a non-functional mutated TonE response element. The level of promoter activity during isotonia was rather low and, thus, hypotonia evoked only a minor reduction in the reporter gene activity (Fig. 2). On the other hand, hypertonia significantly increased the reporter activity in both HCS-2/8 cells (Fig. 2a) and bovine primary chondrocytes (Fig. 2b). Evidence for the specificity of the response was indicated by the finding that the mutated TonE response element did not activate the reporter gene (Fig. 2). Cyclic cellular stretching, which also strains the plasma membrane, had no effect on the activity of the taurine transporter promoter gene activity (Fig. 3a) or gene expression levels (Fig. 3b).

The relative taurine transporter mRNA level decreased both in the HCS-2/8 chondrosarcoma cells and the bovine primary chondrocytes cultured in hypotonic medium (Fig. 4). In the bovine chondrocytes, the level of taurine transporter expression remained elevated in hypertonia throughout the whole 96-h treatment (Fig. 4b), while in the human HCS-2/8 chondrosarcoma cells, the mRNA expression level peaked significantly only at 8 h (Fig. 4a).

Taurine supplementation diminishes the hypertonia-induced cell death of HCS-2/8 cells

Changes in tissue osmolarity threaten the vitality of the cells. However, chondrocytes must be able to adapt to the high osmolarity environment present in cartilage, and even relatively large changes in osmolarity do not markedly affect their vitality. In this study, the 600 mOsm/l medium exerted a small, but significant, increase in lactate dehydrogenase release, indicative of a minor extent of cell death (Fig. 5). In the presence of 20 mM taurine supplementation in the medium, no statistically significant difference was noted in the anisotonic cultures (Fig. 5), suggesting that taurine supplement had a certain protective effect against the cell death.

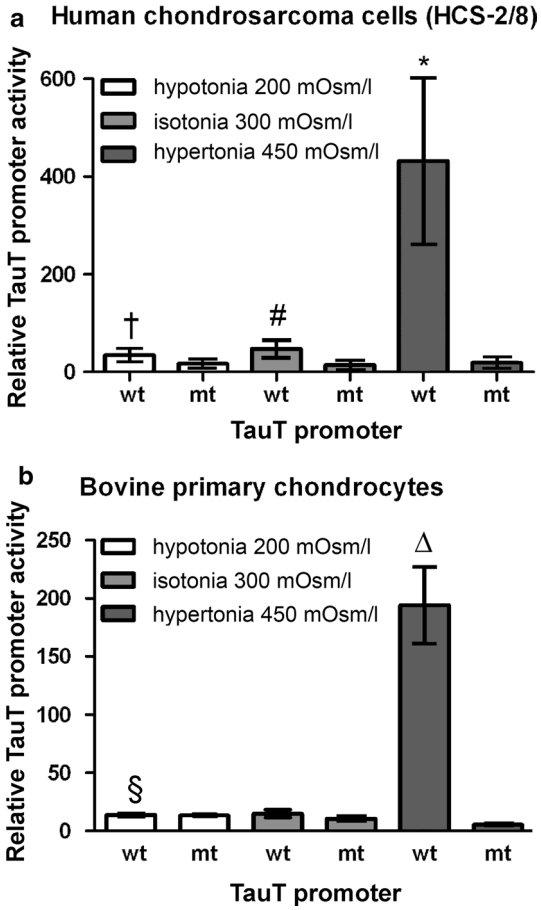

Fig. 2 Taurine transporter promoter activity was analyzed using the luciferase reporter gene assay. Plasmids containing rat wild-type taurine transporter promoter and the one with mutated TonE tonicity response element was transfected into **a** human HCS-2/8 cells and **b** bovine primary chondrocytes together with Renilla luciferase reporter plasmid [$n = 3$, mean (95 % CIs)]. The firefly luciferase activities were normalized against the Renilla luciferase activities. Abbreviation: wt, wild-type promoter; mt, mutated promoter. Statistically significant differences: †hypotonia vs. hypertonia, $p = 0.037$; #hypotonia vs. isotonia, $p = 0.008$; *hypertonia vs. isotonia, $p = 0.040$; §hypotonia vs. hypertonia, $p = 0.009$; Δhypertonia vs. isotonia, $p = 0.008$

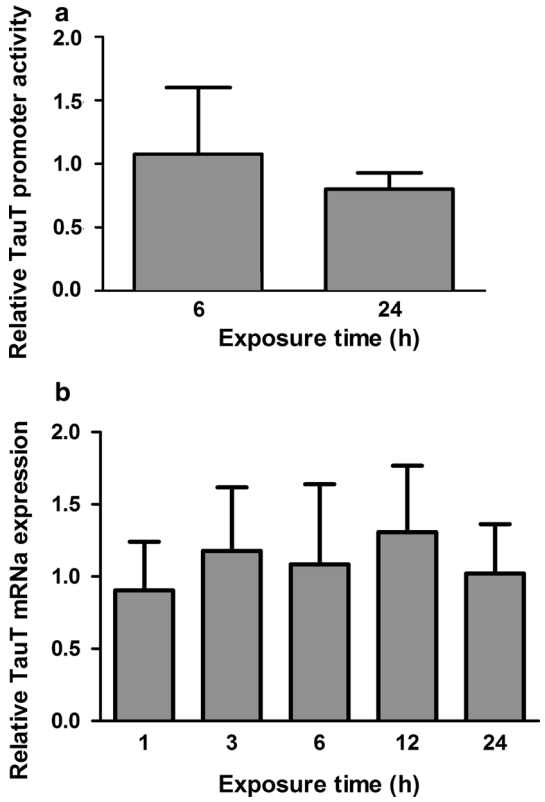

Fig. 3 The effect of cyclic stretching on **a** taurine transporter promoter activity [$n = 4$ mean (95 % CIs)] and **b** gene expression [$n = 3$, mean (95 % CIs)] was analyzed. No statistically significant differences could be observed in either analyses. Non-stretched promoter activity and gene expression was used as normalization samples

The taurine transporter-enhanced green fluorescent protein fusion protein locates at the plasma membrane

The coding sequence of the human taurine transporter mRNA was cloned into a commercially available mammalian expression vector to examine the localization of the taurine transporter-enhanced green fluorescent protein fusion protein in the transfected cells. Confocal microscopy revealed an almost exclusive location of the fusion protein in the plasma membrane of the HCS-2/8 cells (Fig. 6a), although a number of filopodia appeared at the adherence level of the cells (Fig. 6b). Three-dimensional reconstruction of the optical sections revealed that the cells displayed a rather spherical shape (Fig. 6c). In the majority of cells, fluorescence was also visible in a distinctive area close to the nucleus, which we assume represents the presence of the nascent fusion protein in the Golgi apparatus. The location of the nucleus and the apparent Golgi are indicated in the Fig. 6a.

Since the taurine transporter was initially identified in the kidney tubule cells, we investigated the localization of taurine transporter fusion protein also in kidney tubular epithelial cell line MDCK. Those micrographs revealed a surprising number and prominent appearance of filopodia in the MDCK cells (Fig. 6d). Approximately, 70° branching of these structures suggests that actin/WASP complexes exert a major impact on the assembly of the extensions (Fig. 6d).

As the taurine transporter is a plasma membrane protein, it was interesting to determine in which plasma membrane compartment it was located in polarized cells. Therefore, confluent MDCK cell cultures, which stably expressed taurine transporter-EGFP fusion protein were imaged with a confocal microscope. Cells stably expressing EGFP were used as a control. The control cells in monolayers displayed an abundant cytosolic location of the fluorescent protein (Fig. 7a), while the fusion protein exhibited prominent staining in the

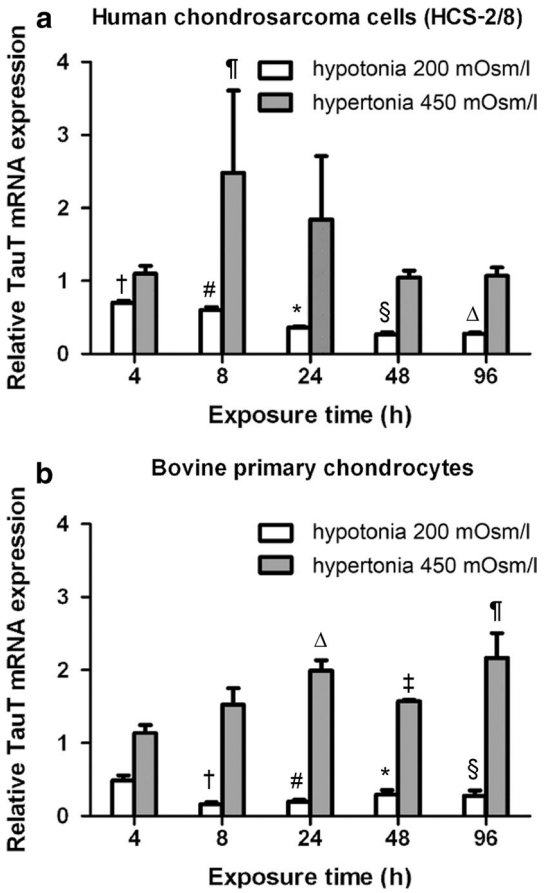

Fig. 4 Time course of taurine transporter mRNA expression in hypotonia and hypertonia [$n = 3$, means (95 % CIs)]. Taurine transporter vs. RPLP0 expression levels in **a** HCS-2/8 chondrosarcoma cell and **b** bovine primary chondrocytes after the hypo- and hypertonic treatments were normalized against the level of isotonia at the beginning of the experiment. **a** Statistically significant differences vs. start point in hypotonia: $^{\dagger}4$ h, $p = 0.007$; $^{\#}8$ h, $p = 0.011$; *24 h, $p = 0.001$; $^{\S}48$ h, $p = 0.002$; $^{\Delta}96$ h, $p = 0.001$. Statistically significant differences vs. start point in hypertonia: $^{\P}8$ h, $p = 0.049$. **b** Statistically significant differences vs. start point in hypotonia: $^{\dagger}8$ h, $p = 0.030$; $^{\#}24$ h, $p = 0.032$; *48 h, $p = 0.024$; $^{\S}96$ h, $p = 0.019$. Statistically significant differences vs. start point in hypertonia: $^{\Delta}24$ h, $p = 0.038$; $^{\ddagger}48$ h, 0.053; $^{\P}96$ h, $p = 0.002$

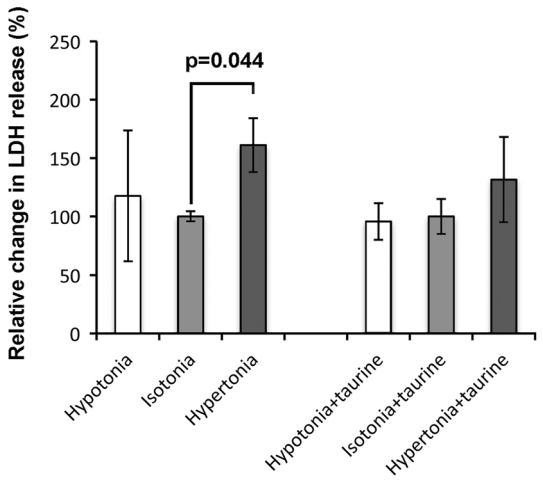

Fig. 5 Release of lactate dehydrogenase in osmotically challenged human chondrosarcoma cells. The cells were cultured in 200, 300 and 600 mOsm/l medium in the absence or presence of 20 mM taurine supplementation for 6 h. The extent of hypertonia-induced cell death lost statistical significance in taurine-supplemented cultures [$n = 3$, means (95 % CIs)]

lateral part of the plasma membrane (Fig. 7b). Since monolayer cultures may have a deficient basement membrane, we also cultivated the stably transfected cell lines in Matrigel™-generated three-dimensional matrices. Cysts in the control cultures showed intense fluorescence in their cytosols (Fig. 7c), while TauT-EGFP overexpressing polarized cells had the most intense fluorescence on the lateral side of the cell (Fig. 7d).

Taurine in chondrogenic and osteogenic differentiation

Taurine was included in the chondrogenic medium to determine whether it could improve the chondrogenesis of the human mesenchymal stem cells. However, the results obtained from three donors did not give any consistent outcome. Mesenchymal stem cells from one donor showed a high potential for chondrogenesis, and in this case, the chondrogenesis appeared to be even more profound in the presence of taurine. However, other donors' cells did not benefit from taurine supplementation, while taurine supplementation in the chondrogenesis assay with the third donor's cells generated such a small chondrogenic pellet that the sample was lost during its histological processing.

Osteogenesis in the presence of taurine was investigated, since a previous study (Yuan et al. 2006) had suggested that taurine supplementation could increase alkaline phosphatase activity and the osteocalcin content in MG-63 osteoblasts. In this study, the levels of alkaline phosphatase activity increased constantly throughout the 4-week differentiation period. However, taurine supplementation did not appear to have any major effect over regular osteogenic medium in inducing the enzyme activity (results not shown). Staining of fixed cells for alkaline phosphatase activity for a differentiation period of 2 and 4 weeks gave a similar result. The mRNA expression levels of Cbfa1/RUNX2, an essential transcription factor for osteoblastic differentiation, appeared to be slightly elevated in osteogenic samples, but no consistent effect of taurine could be observed (data not shown). No effect in the expression levels of procollagen α_1(I) and procollagen α_2(I) could be found either (data not shown). Since we could not find that taurine would benefit osteogenesis, we analyzed the effect of taurine on alkaline phosphatase activity in MG-63 osteoblasts, but we could not confirm the increased activity that has been previously described (Yuan et al. 2006).

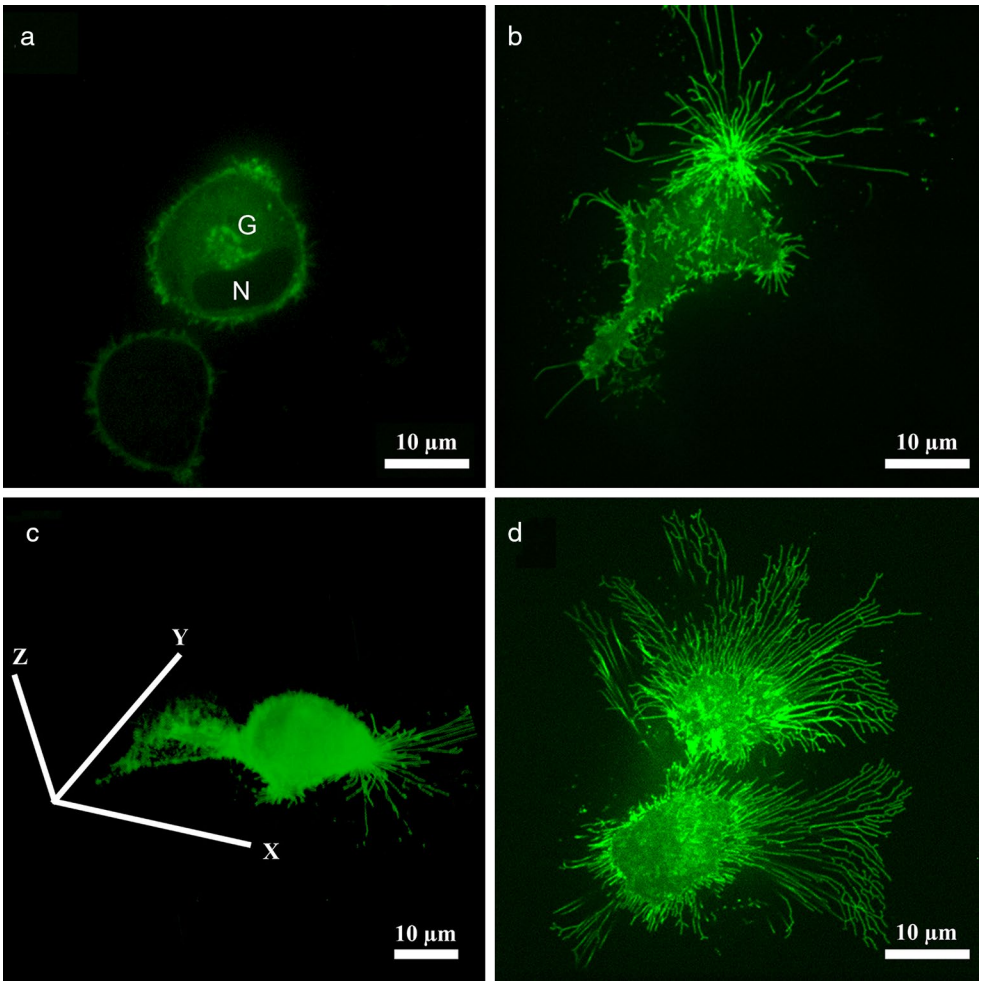

Fig. 6 Taut-EGFP-N1 plasmid-transfected HCS-2/8 (**a**–**c**) and MDCK (**d**) cells. An optical section from the middle part of the cell clearly shows the dominant location of fluorescence in the plasma membrane (**a**). Numerous filopodia were present close to the cell attachment plane (**b**). This is also clearly visible in the three-dimensional reconstruction of the cell from the stack of optical sections (**c**). An image of a transfected MDCK cell shows the numerous filopodia with 70° branching points (**d**). *G* the Golgi apparatus, *N* nucleus

Discussion

The role of the taurine transporter in chondrocytes is a poorly investigated topic. This is the first study to examine its expression and transcriptional activation under anisotonic conditions. As far as we are aware, this is also the first study using fluorescently labeled taurine transporter fusion protein to reveal its cellular location not only in chondrosarcoma cells, but also in polarized epithelial kidney tubule cells.

Taurine efflux in isolated chondrocytes and in cartilage explants was described already in 1995 (Hall 1995), but only a few studies of taurine transport in chondrocyte or cartilage have been published since then. In here, we can demonstrate that changes in osmolarity can regulate the expression level of the taurine transporter. The regulatory element appears to be located in the TonE, since the mutated TonE did not induce any activation of transcription. These results are in line with those obtained in nucleus pulposus cells (Tsai et al. 2007).

Taurine has been reported to be able to enhance cell viability in retinal ganglion cells (Froger et al. 2013). Since its function is associated with changes in tonicity, we tested whether taurine would have any role as a protector against the osmolarity challenges faced by chondrocytes. We observed that chondrocytes can tolerate rather high differences in osmolarity, since these changes evoked only a low percentage of cell death. However, hypertonic conditions at 600 mOsm/l revealed a statistically significant level of cell death, and this could be partially rescued by the presence of 20 mM taurine in the cell culture medium.

Due to its function in taurine transport, the location of the taurine transporter would be intuitively sited in the plasma membrane. This study confirms that the taurine transporter is rather evenly distributed in this non-polarized

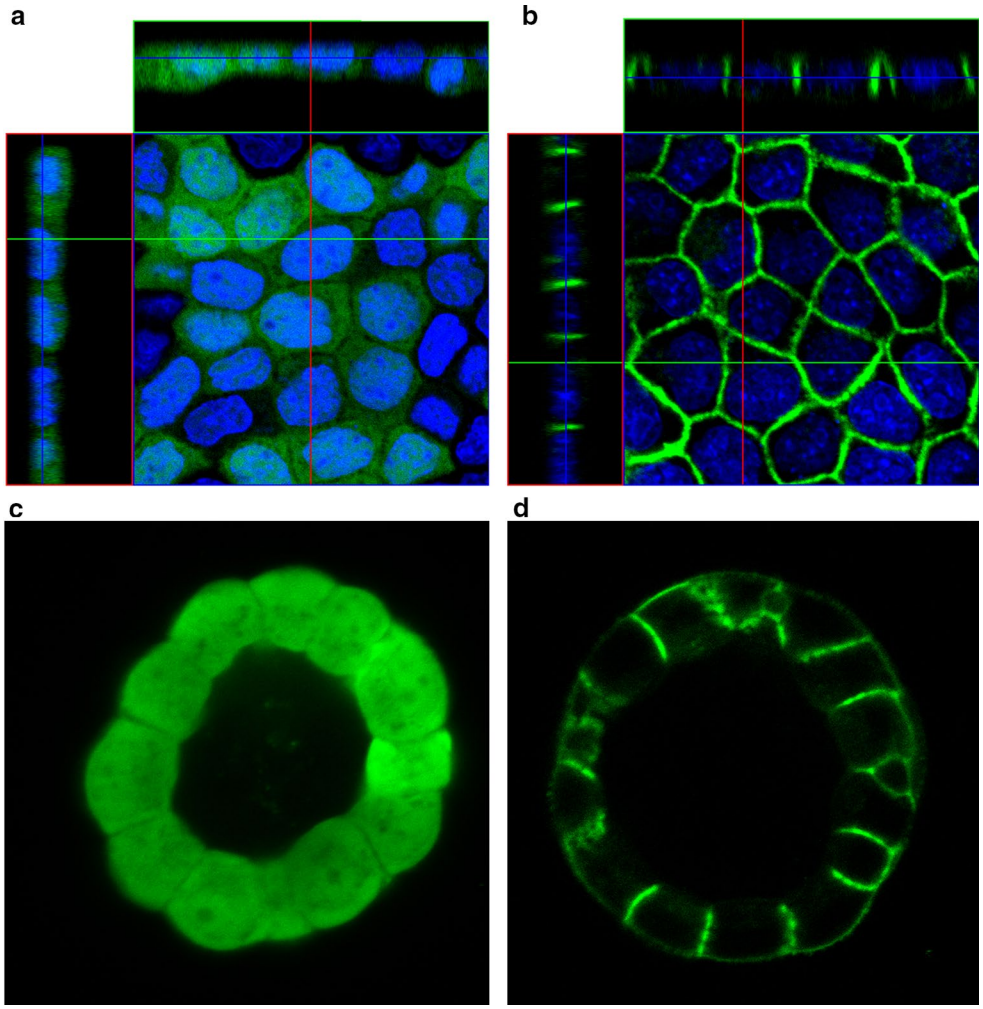

Fig. 7 The localization of taurine transporter-EGFP fusion protein in stably transfected polarized MDCK cells. The EGFP-expressing control cells show intense staining in the cytosol of confluent monolayer cultures (**a**), while the fusion protein has the most intense staining on the lateral side of cells (**b**). The vertical views from the planes are shown in the *insets*, while *colored lines* map their location in the larger images. Similar findings were evident in cysts for control EGFP (**c**) and fusion protein expressing cells (**d**) formed in Matrigel (color figure online)

cell. In addition, our results suggest that in polarized MDCK cells it is concentrated mainly on the lateral side of the cell. It remains to be investigated whether this is specific for MDCK cells. Since the basolateral plasma membrane is generally considered to allow free planar movement of proteins, our findings suggest that some specific interactions may be involved in integrating the taurine transporter predominantly to the lateral side. The taurine transporter's even distribution in the plasma membrane of the chondrocytes can be considered to be advantageous for its efficient function over the whole plasma membrane area under the demanding osmotic conditions of the cartilage tissue. On the other hand, localization of taurine transporter may be different inside the chondrons, which have plasma membrane domains facing either the lacuna border or the adjacent chondrocyte.

The role of taurine in chondrogenic and osteogenic differentiation was also investigated. No definitive role could be designated in either differentiation pathway. In one donor out of three, taurine could induce better chondrogenesis of bone marrow-derived mesenchymal stem cells, but there was extensive variation between donors. The importance of taurine for osteogenesis in MG-63 osteoblasts could not be confirmed, although one previous study had reported increased alkaline phosphatase activity and an elevated osteocalcin content in osteoblasts after taurine supplementation (Yuan et al. 2006). It is well known that the differentiation potential of various osteoblastic cell lines differs, and the use of different cell lines could explain the observed differences, particularly when originally non-differentiated mesenchymal stem cells are used.

In conclusion, this study describes the gene activation and transcriptional regulation of the taurine transporter, revealing its location in non-polarized and polarized cells, and speculates on its role in chondrogenic and osteogenic differentiation. This work also provides novel information on the function of taurine transporter.

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Conflict of interest The authors declare no conflict of interest.

References

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Conte Camerino D, Tricarico D, Pierno S, Desaphy JF, Liantonio A, Pusch M, Burdi R, Camerino C, Fraysse B, De Luca A (2004) Taurine and skeletal muscle disorders. Neurochem Res 29:135–142

Fort P, Marty L, Piechaczyk M, el Sabrouty S, Dani C, Jeanteur P, Blanchard JM (1985) Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res 13:1431–1442

Froger N, Jammoul F, Gaucher D, Cadetti L, Lorach H, Degardin J, Pain D, Dubus E, Forster V, Ivkovic I, Simonutti M, Sahel JA, Picaud S (2013) Taurine is a crucial factor to preserve retinal ganglion cell survival. Adv Exp Med Biol 775:69–83

Hall AC (1995) Volume-sensitive taurine transport in bovine articular chondrocytes. J Physiol 484:755–766

Hall AC, Bush PG (2001) The role of a swelling-activated taurine transport pathway in the regulation of articular chondrocyte volume. Pflugers Arch 442:771–781

Han X, Budreau AM, Chesney RW (2000) Cloning and characterization of the promoter region of the rat taurine transporter (TauT) gene. Adv Exp Med Biol 483:97–108

Hoffmann EK, Lambert IH, Pedersen SF (2009) Physiology of cell volume regulation in vertebrates. Physiol Rev 89:193–277

Holmdahl R, Rubin K, Klareskog L, Larsson E, Wigzell H (1986) Characterization of the antibody response in mice with type II collagen-induced arthritis, using monoclonal anti-type II collagen antibodies. Arthritis Rheum 29:400–410

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

Ito T, Fujio Y, Hirata M, Takatani T, Matsuda T, Muraoka S, Takahashi K, Azuma J (2004) Expression of taurine transporter is regulated through the TonE (tonicity-responsive element)/TonEBP (TonE-binding protein) pathway and contributes to cytoprotection in HepG2 cells. Biochem J 382:177–182

Ito T, Muraoka S, Takahashi K, Fujio Y, Schaffer SW, Azuma J (2009) Beneficial effect of taurine treatment against doxorubicin-induced cardiotoxicity in mice. Adv Exp Med Biol 643:65–74

Jeon SH, Lee MY, Kim SJ, Joe SG, Kim GB, Kim IS, Kim NS, Hong CU, Kim SZ, Kim JS, Kang HS (2007) Taurine increases cell proliferation and generates an increase in [Mg2+]i accompanied by ERK 1/2 activation in human osteoblast cells. FEBS Lett 581:5929–5934

Kaitainen S, Mähönen AJ, Lappalainen R, Kröger H, Lammi J, Qu MC (2013) TiO_2 coating promotes human mesenchymal stem cell proliferation without the loss of their capacity for chondrogenic differentiation. Biofabrication 5:025009

Kevresan S, Kuhajda K, Kandrac J, Fawcett JP, Mikov M (2006) Biosynthesis of bile acids in mammalian liver. Eur J Drug Metab Pharmacokinet 31:145–156

Koide M, Okahashi N, Tanaka R, Kazuno K, Shibasaki K, Yamazaki Y, Kaneko K, Ueda N, Ohguchi M, Ishihara Y, Noguchi T, Nishihara T (1999) Inhibition of experimental bone resorption and osteoclast formation and survival by 2-aminoethanesulphonic acid. Arch Oral Biol 44:711–719

Lambert IH (2004) Modulation of volume-sensitive taurine release from NIH3T3 mouse fibroblasts by reactive oxygen species. Adv Exp Med Biol 559:369–378

Lang F (2007) Mechanisms and significance of cell volume regulation. J Am Coll Nutr 26:613S–623S

Marcinkiewicz J, Kontny E (2012) Taurine and inflammatory diseases. Amino Acids 46:7–20

Neidlinger-Wilke C, Wilke HJ, Claes L (1994) Cyclic stretching of human osteoblasts affects proliferation and metabolism: a new experimental method and its application. J Orthop Res 12:70–78

Qu CJ, Pöytäkangas T, Jauhiainen M, Auriola S, Lammi MJ (2009) Glucosamine sulphate does not increase extracellular matrix production at low oxygen tension. Cell Tissue Res 337:103–111

Qu C, Lindeberg H, Ylärinne JH, Lammi MJ (2012) Five percent oxygen tension is not beneficial for neocartilage formation in scaffold-free cell cultures. Cell Tissue Res 348:109–117

Qu C, Puttonen KA, Lindeberg H, Ruponen M, Hovatta O, Koistinaho J, Lammi MJ (2013) Chondrogenic differentiation of human pluripotent stem cells in chondrocyte co-culture. Int J Biochem Cell Biol 45:1802–1812

Ripps H, Shen W (2012) Review: taurine: a "very essential" amino acid. Mol Vis 18:2673–2686

Takigawa M, Tajima K, Pan HO, Enomoto M, Kinoshita A, Suzuki F, Takano Y, Mori Y (1989) Establishment of a clonal human chondrosarcoma cell line with cartilage phenotypes. Cancer Res 49:3996–4002

Tsai TT, Guttapalli A, Agrawal A, Albert TJ, Shapiro IM, Risbud MV (2007) MEK/ERK signaling controls osmoregulation of nucleus pulposus cells of the intervertebral disc by transactivation of TonEBP/OREBP. J Bone Miner Res 22:965–974

Turunen SM, Lammi MJ, Saarakkala S, Koistinen A, Korhonen RK (2012) Hypotonic challenge modulates cell volumes differently in the superficial zone of intact articular cartilage and cartilage explant. Biomech Model Mechanobiol 11:665–675

Urban JP (1994) The chondrocyte: a cell under pressure. Br J Rheumatol 33:901–908

Yamori Y, Taguchi T, Hamada A, Kunimasa K, Mori H, Mori M (2010) Taurine in health and diseases: consistent evidence from experimental and epidemiological studies. J Biomed Sci 17(Suppl 1):S6. doi:10.1186/1423-0127-17-S1-S6

Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J Exp Biol 208:2819–2830

Yuan LQ, Xie H, Luo XH, Wu XP, Zhou HD, Lu Y, Liao EY (2006) Taurine transporter is expressed in osteoblasts. Amino Acids 31:157–163

Yuan LQ, Liu W, Cui RR, Wang D, Meng JC, Xie H, Wu XP, Zhou HD, Lu Y, Liao EY (2010) Taurine inhibits osteoclastogenesis through the taurine transporter. Amino Acids 39:89–99

Zhang LY, Zhou YY, Chen F, Wang B, Li J, Deng YW, Liu WD, Wang ZG, Li YW, Li DZ, Lv GH, Yin BL (2011) Taurine inhibits serum deprivation-induced osteoblast apoptosis via the taurine transporter/ERK signaling pathway. Braz J Med Biol Res 44:618–623

Zhou C, Zhang X, Xu L, Wu T, Cui L, Xu D (2014) Taurine promotes human mesenchymal stem cells to differentiate into osteoblast through the ERK pathway. Amino Acids 46:1673–1680