Influence of 1α, 25-dihydroxyvitamin D₃ [1, 25(OH)₂D₃] on the expression of Sox 9 and the transient receptor potential vanilloid 5/6 ion channels in equine articular chondrocytes

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Abstract

Background: Sox 9 is a major marker of chondrocyte differentiation. When chondrocytes are cultured in vitro they progressively de-differentiate and this is associated with a decline in Sox 9 expression. The active form of vitamin D, 1, 25 (OH)₂D₃ has been shown to be protective of cartilage in both humans and animals. In this study equine articular chondrocytes were grown in culture and the effects of 1, 25 (OH)₂D₃ upon Sox 9 expression examined. The expression of the transient receptor potential vanilloid (TRPV) ion channels 5 and 6 in equine chondrocytes in vitro, we have previously shown, is inversely correlated with de-differentiation. The expression of these channels in response to 1, 25 (OH)₂D₃ administration was therefore also examined.

Results: The active form of vitamin D (1, 25 (OH)₂D₃) when administered to cultured equine chondrocytes at two different concentrations significantly increased the expression of Sox 9 at both. In contrast 1, 25 (OH)₂D₃ had no significant effect upon the expression of either TRPV 5 or 6 at either the protein or the mRNA level.

Conclusions: The increased expression of Sox 9, in equine articular chondrocytes in vitro, in response to the active form of vitamin D suggests that this compound could be utilized to inhibit the progressive de-differentiation that is normally observed in these cells. It is also supportive of previous studies indicating that 1α, 25-dihydroxyvitamin D₃ can have a protective effect upon cartilage in animals in vivo. The previously observed correlation between the degree of differentiation and the expression levels of TRPV 5/6 had suggested that these ion channels may have a direct involvement in, or be modulated by, the differentiation process in vitro. The data in the present study do not support this.

Keywords: Cartilage, Vitamin D, Sox 9, TRPV, Chondrocyte

Background

The active metabolite of vitamin D, 1, 25 (OH)₂D₃ has been reported to be involved in the regulation of cellular functions such as differentiation, proliferation, and the immune system [1]. Similar to other steroids, 1, 25 (OH)₂D₃ exerts physiological actions at two levels by genomic and non-genomic mechanisms. The genomic mechanism exerts its effect on the cells by binding to specific receptors known as vitamin D receptors, which in turn bind to retinoid X receptors to form heterodimeric complexes [2]. These heterodimeric complexes in turn bind to VDREs (vitamin D responsive elements) of the upstream region of the responsive gene leading to activation/suppression of the target gene. In contrast, the non-genomic effect of 1, 25 (OH)₂D₃ is mediated though plasma membrane receptors of the target cells which are involved in ion channel activity regulation and also activate signal transduction pathways [3,4]. Exogenous 1, 25 (OH)₂D₃ in mice increased chondrocyte proliferation as well as enhancing...
cartilage matrix mineralization [5]. The profound effects of vitamin D on calcium metabolism are well established and it has been reported that it can protect against cartilage loss in osteoarthritis in humans and rats [6].

Sox 9 (Sry-type high mobility group domain) transcription factor is expressed in articular chondrocytes, central nervous and urogenital systems [7,8]. It is expressed in all primordial cartilage tissues during embryogenesis [8,9] and functions to induce differentiation of stem cells to chondrocytes [10], regulate cartilage development and phenotypic maintenance during embryonic development [11]. Studies indicate that Sox 9 is involved in chondrocyte differentiation by regulating the expression of cartilage-specific genes such as collagen type II [8,12], XIa2 [13], aggrecan [14], collagen link protein [15], cartilage oligomeric matrix protein and Cd-rap [16]. It has been shown that Sox 9 expression is maintained in articular chondrocytes and reduced in osteoarthritic chondrocytes [17].

Haploinsufficiency of Sox 9 results in campomelic dysplasia, a severe syndrome resulting in inadequate cartilage formation during development leading to severe dwarfism [18]. In chimaeric mice, Sox 9−/− the prechondrogenic mesenchyme cells were prevented from differentiating into chondrocytes and lost their ability to express chondrocyte specific genes [19,20]. Sox 9 expression is also significantly reduced in osteoarthritic cartilage compared to normal healthy cartilage [17,21].

In vitro propagation of articular chondrocytes results in de-differentiation that is characterized by gradual loss of chondrocytic phenotype and acquisition of a fibroblastic phenotype [22] which is associated with a rapid decline in Sox 9 expression [23]. We have previously shown in equine chondrocytes that there is an association between the degree of de-differentiation and the expression and the transient receptor potential vanilloid (TRPV) channels 5 and 6 [24].

The transient receptor potential (TRP) superfamily is a non-selective cation ion channels with relative calcium selectivity. The TRPV sub-family is divided into two groups. TRPV1-4 channels are non-selective ion channels with modest permeation to calcium. This group can be activated by different stimuli such as heat/cold, chemical/mechanical stresses and binding to second messengers [25,26]. The other group comprises of TRPV5 and TRPV6 channels that are highly calcium selective and tightly regulated by cytosolic Ca2+ concentration [27,28]. The TRPV5 channel is implicated in Ca2+ reabsorption from the kidney, whereas TRPV6 channel is involved in Ca2+ absorption in the intestine [29]. Expression of both channels in human articular chondrocytes at mRNA level has been reported [30]. We also demonstrated their expression at the protein levels in equine articular chondrocytes (EAC) [24]. A correlation between the expression of TRPV5/6 channels and administered 1, 25 (OH)2D3 concentration has also reported in intestinal endothelial cells [31,32], renal cells [33], osteoblasts [34,35]. Calbindin-D9K is a cytosolic Ca2+ binding protein, a member of cellular proteins found in the cells with high affinity for Ca2+ ions. Calbindin-D9K knockout mice models demonstrated that 1, 25 (OH)2D3 intake increased the expression of both channels [36]. The aim of the present study was to examine the effects of 1, 25 (OH)2D3 on the expression of Sox 9 and TRPV 5 and 6 in cultured equine chondrocytes.

Methods
Chondrocyte isolation
Articular chondrocytes were isolated from equine articular cartilage removed from load bearing synovial joints (metacarpophalangeal joints) of skeletally mature animals obtained on the day of slaughter from a local abattoir; these animals were euthanized for purposes other than research. All experiments were performed with local institutional ethical approval (University of Nottingham, School of Veterinary Medicine and Science Ethical Committee), in strict accordance with national guidelines. Articular cartilage slices were collected in serum free DMEM medium supplemented with 2% antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin) (Invitrogen, UK). Cartilage slices were washed in phosphate buffer saline (PBS) supplemented with 10% antibiotics (50 U/ml penicillin and 50 μg/ml) for 30 min with agitation, followed by enzymatic digestion in freshly prepared 0.1% (v/w) collagenase type I from clostridium histolyticum enzyme dissolved in serum free medium supplemented with 2% antibiotics at relative humidity of 95%, 5% CO2, 37°C for 18 h. Undigested cartilage debris were removed from the cell/medium suspension by filtering the mixture through a nylon filter strainer of 70 μm pore size (BD Bioscience, Europe). Supernatant was spun to isolate the chondrocytes, followed by three washes using PBS containing 10% antibiotics. Finally cells were suspended in DMEM containing 2% antibiotics and 10% FCS and cultured at 37°C, 95% humidity and 5% CO2 until confluent. Cells viability was assessed by the trypan blue exclusion test (Sigma Aldrich, UK).

Vitamin D treatment
To explore the influence of the active form of vitamin D (1, 25, α-dihydroxy vit D) on equine articular chondrocytes, cells were cultivated at 2 10⁵ cells/well until sub-confluence. Chondrocytes were treated with different concentrations of 1, 25 (OH)2D3 (1 10⁻⁶ and 1 10⁻¹²) [37] in serum free-medium for 24 h. The concentration of DMSO was below 0.1%. At the end of 1, 25 (OH)2D3 treatment, cells were washed three times sterile PBS and whole cell lysate was collected for protein expression using western blotting.
Western blotting
Total cellular protein lysate was isolated using radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 50 mM Tris HCl, pH 7.5, 5 mM ethylene glycol tetraacetic acid (EGTA), 1% Triton, 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulphate) supplemented with phosphatase and protease inhibitors cocktail (Roche Diagnostic, Mannheim, Germany) on ice. Protein concentration was quantified by the Bradford method. 25 μg of total protein lysate was mixed with sample buffer (0.5 M Tris HCl, pH 6.8, 100% glycerol, 20% SDS, 0.5% bromophenol blue and 5% β-mercaptoethanol), separated on a 4/1-% polyacrylamide gel, then electrically transferred to PVDF membrane (Invitrogen, UK) by semi-dry apparatus (Bio-Rad, UK). Transferred proteins were blocked for non-specific binding in 5% (w/v) non-fat milk diluted in Tris base buffer saline with 0.1% tween 20 (TBS-T), followed by incubation with designated antibodies overnight. Membranes were washed five times in TBST, followed by 1 h incubation with goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) (Dako, UK) secondary antibody at room temperature. Finally, five washes were carried out for 5 min each followed by developing the membrane with the Amersham ECL western blot enhanced chemiluminescence kit (GE Healthcare, UK) and visualized by exposing to X-ray film.

Quantitative PCR
The quantitative real time PCR was carried out using a LightCycler® 480 PCR System (Roche Diagnostics) using SYBR green DNA-binding fluorescent dye. 20 μl reactions were made in an optical 96-well reaction plate in triplicate and contained: a mixture of template cDNA (5 μl), sense and anti-sense gene specific primers (0.8 μl) (20 pmol), SYBR Green detection reagent and 3.4 μl RNA-free water. Reaction plate was sealed with ABI-prism optical adhesive cover, and spun at 2000 rpm for 2 min. The expression of target genes were normalised against GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and HPRT (Hypoxanthine-guanine phosphoribosyltransferase) using comparative cycle of threshold (Ct) value method.

Statistical analysis
Data values are presented as the mean ± SEM. Each experiment was performed in triplicate. The relative expression on the graphs represents the mean of a combination of three experiments. The differences between animals were analyzed utilizing Students t-test followed by the Bonferroni correction. P values less than or equal to 0.05 were considered statically significant.

Results and discussion
The active form of vitamin D (1, 25 (OH)2D3) has been shown to play an important role in Ca2+ homeostasis. Intracellular Ca2+ concentration is involved in several chondrocyte functions including ECM biosynthesis. This study was designed to explore the possible influence of the active form of vitamin D (1, 25 (OH)2D3) treatment on the expression of Sox 9 and members of the TRP subfamily that are known as epithelial Ca2+ channels (TRPV5 and TRPV6 channels).

The influence of two doses (1 × 10−9 and 1 × 10−12) of 1, 25 (OH)2D3 treatment for 24 h on the expression of the transcription factor (Sox 9) at the protein level in equine chondrocytes was examined. Incubation of chondrocytes for 24 h with 1 × 10−12 of 1, 25 (OH)2D3 significantly increased the expression of Sox 9 by nearly 3 fold, whereas treatment with 1 × 10−9 of 1, 25 (OH)2D3 induced more than 2 fold increase (P < 0.001) compared to the non-treated cells (Figure 1).

In chondrocytes, the mechanisms by which Sox 9 expression is regulated is of great interest due to the critical role played by this transcription factor in controlling the chondrocyte phenotype. The findings of the present study shows an elevation of Sox 9 protein in response to treatment with 1, 25 (OH)2D3. Isolation of chondrocytes results in their de-differentiation and loss of chondrocyte phenotype to fibroblast phenotype [22]. Recent studies have reported changes in the pattern of expression of some proteins during the course of chondrocytes de-differentiation. Cultivation of de-differentiated chondrocytes in alginate gel results in restoring the expression of transcription factor Sox 9 and chondrocyte differentiation markers such as collagen type II and aggrecan [38]. The current study indicated that treatment of cultured chondrocytes with 1, 25 (OH)2D3 results in restoring of the transcription factor Sox 9, which could indicate chondrocyte re-differentiation and restoration of the chondrocyte phenotype.

The physiological activities of Sox 9 protein were demonstrated by mouse genetic studies which indicated the importance of Sox 9 expression level in determining chondrocyte phenotype during development [39,40]. These studies demonstrated that chondrocyte differentiation and cartilage development were severely affected by knock-out or knock-in of a single allele of Sox 9. Moreover, the campomelic dysplasia is a genetic disorder characterized by multiple developmental abnormalities including cartilage induced by haplo-insufficiency of Sox 9 [18].

In human normal articular chondrocytes, Sox 9 expression was progressively reduced by passage in monolayer cell culture [41]. Moreover its expression is reduced in osteoarthritic chondrocytes compared to the normal articular chondrocytes [23] and has therefore been suggested to contribute in osteoarthritis disease processes by altering the ECM gene expression [42]. Thus, improving the expression of Sox 9 by 1, 25 (OH)2D3 treatment could
provide a new insight into the prevention and/or treatment of osteoarthritis.

In fetal cartilage as well as in mature cartilage, the main role of Sox 9 is to maintain the chondrocyte phenotype in addition to inhibition of hypertrophic chondrocyte differentiation [43]. Therefore, down regulation of Sox 9 expression was suggested to be a precondition for hypertrophic alteration occurring in degenerative cartilage [42,44]. As Sox 9 protein is augmented by 1, 25 (OH)2D3 treatment, this study suggests that 1, 25 (OH)2D3 treatment could be utilized to improve the ECM proteins biosynthesis by enhancing the anabolic activities of articular chondrocytes. After further passages, chondrocytes have been shown to exhibit a more pronounced de-differentiated phenotype and lower levels of Sox 9 [41]. Further studies on the effects of 1, 25 (OH)2D3 upon these cells would be of great interest.

We have previously shown that the expression of both TRPV5 and 6 channels, at the protein level, are inversely related to chondrocyte de-differentiation [24]. In this study we therefore examined the influence of 1, 25 (OH)2D3 treatment on the expression of TRPV5/6 channels on primary equine articular chondrocytes isolated at passage two. The findings of the current study indicated that no statistically significant changes were observed on the expression of TRPV5 and TRPV6 channels at the protein level following treatment with different doses of 1, 25 (OH)2D3 (Figure 2). To ensure that 1, 25 (OH)2D3 had no effect on the expression of these channels we also examined its effect upon their transcript levels but again no significant changes were observed (Figure 3). Previous studies conducted on mouse investigated the effect of 1, 25 (OH)2D3 on epithelial Ca2+ transport including TRPV5 and 6 channels following 1, 25 (OH)2D3 treatment [45,46]. These studies demonstrated that the expression of TRPV5 and TRPV6 channels in kidney and duodenum were stimulated by binding of VDR (vitamin D receptor) at transcriptional level. TRPV6 was reported to have several classes of VDREs (vitamin D response elements) in humans and the mouse [45,47,48].

In the vitamin D receptor knockout mice model, the level of gene expression pattern of the TRPV6 and 5 channels in duodenum is dramatically down-regulated, in contrast no changes are observed in gene expression of other Ca2+ transporters [49,50]. The role of vitamin D in the stimulation of Ca2+ transporters has emerged recently. Therefore, the correlation between 1, 25 (OH)2D3 treatment and Ca2+ transport proteins were investigated...
in several cell types. 1, 25 (OH)2D3 was demonstrated to increase the expression of TRPV6 channel, calbind-D9k and PMCA1b genes in Caco-2 cell lines [36,51]. This finding was not consistent with the current study, where no changes were observed on the expression of either TRPV5 or 6 channels at mRNA level or protein levels. As stated above we have previously observed an inverse correlation between TRPV 5 and 6 levels and differentiation state. The current study suggests that the changes in levels may not be directly linked to the de-differentiation process. This however, needs further study as does the mechanism by which Sox-9 is regulated by 1, 25 (OH)2D3. Ca2+ ions and Ca2+ channels have been implicated in chondrocyte metabolism [52,53]. In vitro studies show that augmented extracellular Ca2+ promotes differentiation toward a hypertrophic phenotype, in contrast Ca2+ reduction improves ECM protein synthesis including collagen type II and aggrecan and delays hypertrophy [54]. Therefore, investigating the effect of the 1, 25 (OH)2D3 on the expression of Ca2+ channels could be considered as a candidate for treatment of joint diseases.

The results of the current study do not, however, suggest an involvement of TRPV5/6 ion channels in the regulation of vitamin D mediated up-regulation of sox-9.

**Figure 2** Expression of TRPV5 and TRPV6 channels in equine articular chondrocytes at the protein level. The changes in expression of TRPV5 (A) and TRPV6 (B) channels following treatment with 1, 25 vit D were assessed by densitometry analysis of western blots and normalized to the expression of housekeeping protein (β-Tubulin). Equine kidney (K) lysate was used as a positive control. Levels of expression at the protein level of the TRPV5 (C) and TRPV6 (D) channels following 24 h of incubation with different doses of 1, 25 (OH)2D3 in addition to the solvent (DMSO). Data presented as a mean ± S.E.
Conclusions

This study examined the effects of the active form of vitamin D (1, 25(OH)2D3) upon the expression of transcription factor Sox 9 and the calcium sensitive TRPV 5 and 6 channels, in equine articular chondrocytes, in vitro. An increased expression of Sox 9, in these chondrocytes, in response to the active form of vitamin D suggests that this compound acts to inhibit the progressive de-differentiation that is normally observed in these cells. It is also supportive of previous studies indicating that 1α, 25-dihydroxyvitamin D3 can have a protective effect upon cartilage in animals in vivo. There was no effect of vitamin D (1, 25(OH)2D3) upon the expression of either TRPV channel at either the protein or mRNA level.

Figure 3 Expression of TRPV5/6 at the mRNA level. End point PCR analysis of TRPV5 (A) and TRPV6 (B) ion channel genes on mRNA isolated from equine articular chondrocytes. The graphs show real-time PCR analysis of TRPV5 (C) and TRPV6 (D) ion channel gene expression levels. Experiments were carried out on equine articular chondrocytes (control) and treated with different doses of 1, 25(OH)2D3. GAPDH and HPRT were included as internal controls. Data presented as a mean ± S.E.
level. We have previously observed a correlation between the degree of differentiation and the expression levels of TRPV 5/6 and suggested that these ion channels may have a direct interaction with the differentiation process in vitro. The data in the present study do not support this.

Competing interests
The authors declare that they have no competing interests.

Authors contributions
ISM and PTL designed the experiment and analyzed the results. ISM carried out cell culture work. Both authors wrote the manuscript and made subsequent changes.

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References
1. Boyan BD, Sylvia VL, Dean DD, Del Toro F, Schwartz Z: Differential regulation of growth plate chondrocytes by 1alpha,25-(OH)2D3 and 24R,25-(OH)2D3 involves cell-maturation-specific membrane-receptor-activated phospholipid metabolism. Crit Rev Oral Biol Med 2002, 13(2):143-154.
2. Tetzlaff LC, Smith SJ, Mawer EB, Woolley DE: Vitamin D receptors in the rheumatoid lesion: expression by chondrocytes, macrophages, and synoviocytes. Ann Rheum Dis 1999, 58(2):118 121.
3. Lehmann B, Meurer M: Vitamin D metabolism. Dermatol Ther 2010, 23(12): 12.
4. Fleet JC: Genomic and proteomic approaches for probing the role of vitamin D in health. Am J Clin Nutr 2004, 80(suppl):1739S-1743S.
5. Xue Y, Karaplis AC, Hendy GN, Goltzman D, Miao D: Vitamin D metabolism. Am J Physiol Regul Integr Comp Physiol 2010, 298(3):R509R548.
6. Castillo EC, Hernandez-Cueto MA, Vega-Lopez MA, Lavalle C, Kouri JB, Chondrogenic differentiation of bovine chondrocytes. Mol Biochem 2017, 10(2):2017 2546.
7. Morais Da Silva S, Hacker A, Harley V, Goodfellow P, Swain A, Lovell-Badge R: Sox9 expression during gonadal development implies a conserved tissue-specific enhancer of the pro SOX9 expression of Sox9 and Col2a1 in cells undergoing chondrogenesis. Am J Physiol 2014, 205(4):1351 1353.
8. Brew CJ, Clegg PD, Boot-Handford RP, Andrew JG, Hardingham TE: Gene expression in human chondrocytes in late osteoarthritis is changed in both fibrillated and intact cartilage without evidence of generalised chondrocyte hypertrophy. Ann Rheum Dis 2010, 69(12):234 240.
9. Wagner T, Wirth J, Meyer J, Zabel B, Held M, Zimmer J, Pasantes J, Bricarelli FD, Keitel J, Hueste E, Wolf U, Tommerup N, Schepers W, Scheier G: Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell 1994, 79(6):1111 1120.
10. Shintani N, Hunziker EB: Sox9 is required for cartilage formation. Nat Genet 1999, 22(3):85 89.
11. Wang J, Tong X, Cao J, Liu X: Sox9 expression and in vitro propagated chondrocytes. Arthritis Rheum 2006, 54(10):3308 3314.
12. Xie WF, Zhang X, Sakano S, Lefebvre V, Sandell LJ: Trans-activation of the mouse cartilage-derived retinoic acid-sensitive protein gene by Sox 9. J Bone Miner Res 1999, 14(5):757 763.
13. Bridge wat LC, Lefebvre V, de Crombrugghe B: Chondrocyte-specific enhancer elements in the Col11a2 gene resemble the Col2a1 tissue-specific enhancer. J Biol Chem 1998, 273(24):14998 15006.
14. Sekiya I, Tsujii K, Koopman P, Watanabe H, Yamada Y, Shinomiyak N, Niuju A, Noda M: Sox9 enhances aggrecan gene promoter/enhancer activity and is up-regulated by retinoic acid in a cartilage-derived cell line, Tc5. J Biol Chem 2000, 275(12):10738 10744.
15. Koy I, Igegawa S: SOX9 dependent and independent transcriptional regulation of human cartilage link protein. J Biol Chem 2004, 279(49):50954 50948.
16. Castellano DL, Mapstone B, Araki Y, Kita H, Kikkawa H, Hirose M, Nakamura A, Tokeshi M, Kim S: Sox9 expression is up-regulated by transforming growth factor beta1 induce the formation of different types of cartilage-joins tissue. J Biol Chem 2007, 282(36):23369 23376.
17. Brew CJ, Clegg PD, Boot-Handford RP, Andrew JG, Hardingham TE: Gene expression in human chondrocytes in late osteoarthritis is changed in both fibrillated and intact cartilage without evidence of generalised chondrocyte hypertrophy. Ann Rheum Dis 2010, 69(12):234 240.
18. Wagnert T, Worth J, Meyer J, Zabel B, Held M, Zimmer J, Pasantes J, Bricarelli FD, Keitel J, Hueste E, Wolf U, Tommerup N, Schepers W, Scheier G: Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell 1994, 79(6):1111 1120.
19. B W, Deng J, Zhang Z, Behringer RR, de Crombrugghe B: Sox 9 is required for cartilage formation. Nat Genet 1999, 22(3):85 89.
20. Hargus G, Kist R, Kramer J, Genstel D, Niestz A, Scherer G, Rohwedel J: Loss of Sox9 function results in defective chondrocyte differentiation of mouse embryonic stem cells in vitro. Int J Dev Biol 2008, 52(4):323 332.
21. Cucchiarini M, Thurn T, Weimer A, Kohn D, Ternerwiger EF, Madry H: Restoration of the extracellular matrix in human osteoarthritic articular cartilage by overexpression of the transcription factor SOX9. Arthritis Rheum 2007, 56(1):158 167.
22. Benya PD, Padilla SR, Nimni ME: Expression of transient receptor potential vanilloid (TRPV) channels in different passages of articular chondrocytes. Int J Mol Sci 2012, 13(4):4433 4445.
23. Leduc IM, Mobasheri A, Loughna PT: Effect of osmotic stress on the expression of TRPV4 and BKCa channels and possible interaction with ERK1/2 and p38 in cultured equine chondrocytes. Am J Phys Cell Physiol 2014, 306(1):C1050 C1057.
24. Cardillo IM, Mobasheri A, Loughna PT: Ectopic expression of Sox9 in human chondrocytic cell line identifies novel genes regulated in primary human chondrocytes and in osteoarthritis. Arthritis Res Ther 2007, 9(5):R107.
25. Vennekens R, Hoenderop JG, Preken J, Stuiver M, Willems PH, Droogmans G, Nilius B, Bindels RJ: Permeation and gating properties of the novel epithelial Ca(2+)-channel. J Biol Chem 2000, 275(6):3963 3969.
26. Nilius B, Vennekens R, Preken J, Hoenderop JG, Bindels RJ, Droogmans G: Whole-cell and single channel monovalent cation currents through the novel rabbit epithelial Ca2+ channel ECaC. J Physiol 2000, 527(Pt 2):239 248.
27. Hoenderop JG, Nilius B, Bindels RJ: Calcium absorption across epithelia. Physiol Rev 2005, 85(1):373 422.
28. Gavenis K, Schumacher C, Schneider U, Eiffeld J, Mollenhauer J, Schmidt-Rohling E: Expression of ion channels of the TRP family in articular chondrocytes from osteoarthritic patients: changes between native and in vitro propagated chondrocytes. Mol Cell Biochem 2009, 321(1 2):135 143.
29. Taparia S, Fleet JC, Peng JB, Wang XD, Wood RJ: 1,25-Dihydroxyvitamin D and 25-hydroxyvitamin D mediated regulation of TRPV6 (a putative epithelial calcium channel) mRNA expression in Caco-2 cells. Eur J Nutr 2006, 45(4):196 204.
30. Teenaponpuntak T, Dorkkam N, Wongdee K, Krishnamra N, Charoenphandhu N: Endurance swimming stimulates transepithelial calcium transport and alters the expression of genes related to calcium absorption in the intestine of rats. Am J Physiol Endocrinol Metab 2009, 296(4):E775 E786.
33. Embark HM, Setianaw I, Poppendieck S, van de Graaf SF, Boehmer C, Palmada M, Wieder T, Gerstberger R, Cohen P, Yun CC, Bindels RJ, Lang F: Regulation of the epithelial Ca2+ channel TRPV6 by the NHE regulating factor NHERF2 and the serum and glucocorticoid inducible kinase isoforms SGK1 and SGK3 expressed in Xenopus oocytes. Cell Physiol Biochem 2004, 14(4):209–212.

34. Zanello LP, Norman AW: Multiple molecular mechanisms of 1 alpha, 25(OH)2-vitamin D3 rapid modulation of three ion channel activities in osteoblasts. Bone 2003, 33(1):71–79.

35. Zhang M, Wang JJ, Chen YJ: Effects of mechanical pressure on intracellular calcium release channel and cytoskeletal structure in rabbit mandibular condylar chondrocytes. Life Sci 2006, 78(21):2480–2487.

36. Lee GS, Jung EM, Choi KC, Oh GT, Jeung EB: Compensatory induction of the TRPV6 channel in a calbindin-D9k knockout mouse: Its regulation by 1,25-Dihydroxyvitamin D3. J Cell Biochem 2009, 108(5):1175–1183.

37. Fernandez-Cancio M, Audi L, Carrascosa A, Toran N, Andaluz P, Esteban C, Granada ML: Vitamin D and growth hormone regulate growth hormone/insulin-like growth factor (GH-IGF) axis gene expression in human fetal epiphyseal chondrocytes. Growth Horm IGF Res 2009, 19(3):232–237.

38. Kumar D, Lacar AB: The transcriptional activity of Sox9 in chondrocytes is regulated by RhoA signaling and actin polymerization. Mol Cell Biol 2009, 29(15):4262–4273.

39. Akiyama H, Lyons JP, Mori-Akiyama Y, Yang X, Zhang R, Zhang Z, Deng JM, Takeko MM, Nakamura T, Behringer RR, de Crombrugghe B: Interactions between Sox9 and beta-catenin control chondrocyte differentiation. Gene Dev 2004, 18(9):1072–1082.

40. Kawakami Y, Rodriguez-Leon J, Ipisua Belmonte JC: The role of TGFbetalases and Sox9 during limb chondrogenesis. Curr Opin Cell Biol 2006, 18(6):723–729.

41. Tew SR, Hardingham TE: Regulation of SOX9 mRNA in human articular chondrocytes involving p38 MAPK activation and mRNA stabilization. J Biol Chem 2006, 281(15):9471–9479.

42. Aigner T, Gebhard PM, Schmid E, Bau B, Harley V, Poschl E: SOX9 expression does not correlate with type II collagen expression in adult articular chondrocytes. Matrix Biol 2003, 22(4):363–372.

43. Bi W, Huang W, Whitworth DJ, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B: Haploinsufficiency of Sox9 results in defective cartilage primordia and premature skeletal mineralization. Proc Natl Acad Sci U S A 2001, 98(12):6698–6703.

44. Gebhard PM, Gehritz A, Bau B, Soder S, Eger W, Aigner T: Quantification of expression levels of cellular differentiation markers does not support a general shift in the cellular phenotype of osteoarthritic chondrocytes. J Orthop Res 2003, 21(1):96–101.

45. Nijenhuis T, Hoenderop JG, van der Kemp AW, Bindels RJ: Localization and regulation of the epithelial Ca2+ channel TRPV6 in the kidney. J Am Soc Nephrol 2003, 14(1):273–2740.

46. Song Y, Peng X, Porta A, Takahata H, Peng JB, Hediger MA, Fleet JC, Christakos S: Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1, 25-Dihydroxyvitamin D3 in the intestine and kidney of mice. Endocrinology 2003, 144(9):3885–3894.

47. Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, Jurutka PW: Molecular mechanisms of vitamin D action. Calcif Tissue Int 2013, 92(2):77–98.

48. Hoenderop JG, Muller D, Van Der Kemp AW, Hartog A, Suzuki M, Ishibashi K, Imai M, Swee P, Willems PH, Van Os CH, Bindels RJ: Calcitriol controls the epithelial calcium channel in kidney. J Am Soc Nephrol 2001, 12(7):1342–1349.

49. Bouillon R, Van Cromphout S, Carmeliet G: Intestinal calcium absorption: molecular vitamin D mediated mechanisms. J Cell Biochem 2003, 88(2):332–339.

50. Van Cromphout SJ, Dewerchin M, Hoenderop JG, Stockmans I, Van Hercke E, Kato S, Bindels RJ, Collen D, Carmeliet P, Bouillon R, Carmeliet G: Duodenal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects. Proc Natl Acad Sci U S A 2001, 98(23):13324–13329.

51. Wood RJ, Tchack L, Tapara S: 1,25-Dihydroxyvitamin D3 increases the expression of the CaT1 epithelial calcium channel in the Caco-2 human intestinal cell line. BMC Physiol 2001, 1:11.

52. Kirsch T, Swoboda B, von der Mark K: Ascorbate independent differentiation of human chondrocytes in vitro: simultaneous expression of types I and X collagen and matrix mineralization. Differentiation 1992, 52(1):89–100.

53. Bonen DK, Schmid TM: Elevated extracellular calcium concentrations induce type X collagen synthesis in chondrocyte cultures. J Cell Biol 1991, 115(4):1171–1178.

54. Koyano Y, Hejna M, Flechtenmacher J, Schmid TM, Thonar EJ, Mollenhauer J: Collagen and proteoglycan production by bovine fetal and adult chondrocytes under low levels of calcium and zinc ions. Connect Tissue Res 1996, 34(3):213–225.

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