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Boron as Boric Acid Induces mRNA Expression of the Differentiation Factor Tuftelin in Pre-Osteoblastic MC3T3-E1 Cells

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Abstract

The effects of boron on the formation and maintenance of mineralized structures at the molecular level are still not clearly defined. Thus, a study was conducted using MC3T3-E1 cells to determine whether boron affected mRNA expressions of genes associated with bone/alveolar bone formation around the teethMC3T3-E1 (clone 4) cells were cultured in media treated with boric acid at concentrations of 0, 0.1, 10, 100, or 1000 ng/ml. Total RNAs of each group were isolated on day 3. Gene expression profiles were determined by using RT^2 Profiler PCR micro-array that included 84 genes associated with osteogenic differentiation. Tuftelin1 mRNA expression was upregulated by all boron treatments. The upregulation was confirmed by quantitative RT-PCR using the tuftelin probe. While 100 ng/ml had no effect on the integrin-α2 (Itga2) transcript and 1 ng/ml boric acid induced Itga2 mRNA expression (2.1-fold), 0.1, 10, and 1000 ng/ml boric acid downregulated the integrin-α2 gene transcript 2.2-, 1.5-, and 2.1-fold respectively. While 0.1 ng/ml boric acid induced BMP6, increased BMP1r mRNA expression (1.5 fold) was observed in 1000 ng/ml boric acid treatment. The findings suggest that boron affects the regulation of the tuftelin1 gene in osteoblastic cells. Further studies are needed to establish that the beneficial actions of boron on alveolar bone and tooth formation and maintenance include an effect on the expression of the tuftelin1 gene.

Keywords Boric acid · Osteoblastic differentiation · Tuftelin · Osteogenesis

Introduction

An increasing number of cell culture, animal, and human studies indicate that boron in nutritional amounts has beneficial health effects. We have found that boron positively regulates genes in MC3T3-E1 cells that are important for bone formation and remodeling [1]. In rabbits fed with a high-energy diet, boron had beneficial effects on bone strength and mineral composition [2]. Rat and mouse studies have shown that boron deficiency impaired injured bone healing by apparently reducing osteogenesis [3, 4]. Boron deficiency decreased alveolar bone remodeling and inhibited bone formation.

The supplements used the studies above showing boron in nutritional or supra nutritional amounts having beneficial effects on bone strength and mineral composition were far from toxic. Intakes of over 100 times nutritional or 10 times supra nutritional are intakes that result in toxicity signs. For example, rats fed with 500 mg boron/kg diet exhibited depressed weight and femur magnesium and zinc, but no significant change in femur calcium and phosphorus concentrations and tibia bone density [5].

Undecalcified histomorphometric evaluation of maturing dental enamel from continuously erupting rat incisor found a reduction in enamel thickness (hypoplasia) [6]. Microchemical characterization by energy-dispersive X-ray spectrometry did not find any alteration in enamel mineralization. In a study histologically examining teeth of rabbits, tooth structure (enamel shape and thickness, dentin, cementum, and pulp) was not significantly affected by boron supplementation [7]. However, micro-CT evaluation found increased alveolar bone mineral

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density when the rabbits were given boron supplements of 10 and 30 mg/kg diet. The difference in the findings may have been the result of the different experimental model used and/or the boron status of the control animals.

An acidic protein that has been found to play a critical role in the initial stages of ectodermal enamel mineralization is tuftelin [8]. During embryonic development, tuftelin was found to become histologically visible in the mouse molar at the E13 stage [9]. Subsequent to 1991, tuftelin was found in other tissues including craniofacial structures [10, 11]. At the E15 embryonic stage, when alveolar bone cells and vascularization appears [12], osteoblasts were found to be positive for tuftelin. Interestingly, unlike teeth, tuftelin in alveolar bone is intracellular, apparently not a matrix component, and changes location during development. Initially, tuftelin is abundant and occurs in the cytoplasm of differentiating/differentiated osteoblasts. At later pre- and post-natal stages, tuftelin-positive osteoblasts decrease with the localization expanding into the perinuclear/nuclear regions.

Bobek et al. (2019) further characterized the spatial-temporal expression of tuftelin in the mineralizing and non-mineralizing tissues of the craniofacial complex in the developing mouse embryo [13]. Two tuftelin mRNA transcripts and a single 64 Kda protein were detected throughout embryonic development. Tuftelin mRNA and protein already were expressed at stage 10.5 before the initiation of tooth formation. Tuftelin was detected in tissues that develop from embryonic ectoderm, ectomesenchyme, and mesoderm origins. This finding in addition to a shift between cytoplasmic and perinuclear/nuclear expression suggests that tuftelin has a role in the regulation of transcription.

The objective of the following study was to determine whether physiological boron influences the mRNA expression related to osteogenic differentiation of MC3T3-E1 cells. Particular attention was given to tuftelin, which has been found to be involved in alveolar bone formation and development.

Material and Methods

Cell Culture

The MC3T3-E1 (subclone 4) cells were kindly provided by Dr. Martha J. Somerman, NIDCR, NIH, USA, and Renny T. Franceschi, University of Michigan, USA. The cells were derived from newborn mouse calvaria [14]. This subclone was chosen because it was suitable for differentiation study [1]. Cells were maintained in an α-MEM medium with 10% fetal bovine serum and 1% penicillin, streptomycin, and L-glutamine (Biological Industries, Israel) in a humidified incubator at 37 °C with 5% CO_2 in the air.

Preparation of Boric Acid

Boric acid (Sigma-Aldrich, Saint Louis, MO, USA) was dissolved in double-distilled water and shaken for 30 min. The solution was adjusted to a pH of 7.0 with NaOH, filtered through 0.22 μm cell culture filter, and sterilized. This stock solution containing 10 μg/mL boric acid was used to make final concentrations of 0.1, 1, 10, 100, and 1000 ng/mL boric acid in the mRNA expression assay medium. These boric acid concentrations provided 0.0175, 0.175, 1.75, 17.5, and 175 ng/mL of boron. Safe/optimum boric acid concentrations were selected according to the studies reported in the literature [1, 15]. Cells were treated with the different concentrations of boric acid every other day.

Gene Analysis

RNA Isolation

Cells at passages 4–7 were used for the gene analysis. Cells were cultured in 60 mm plates at a density of $5 \times 10^4/cm^2$. Total RNAs of cells were isolated on day 3 by using a monophasic solution of phenol and guanidine isothiocyanate (Invitrogen, Carlsbad, CA, USA) [7, 16] and quantified at 260 nm by NanoDrop.

PCR Array Analysis

The gene expression profiles of the MC3T3-E1 cells were evaluated and were determined by using the RT^2 Profiler PCR array system for mouse osteogenesis (PAMM-026 f-12, Qiagen, Germantown, USA) that includes 84 genes related to osteogenic differentiation. The array contains the gene groupings for skeletal development, bone mineral metabolism, cell growth and differentiation, extracellular matrix proteins, and cell adhesion molecules that are shown in Table 1.

PCR Array

Diluted first strand cDNA synthesis reaction (102 μL) was combined with 2448 μL of RT2 qPCR Master Mix (Qiagen, Germantown, USA) to make a final volume of 2550 μL (1275 μL superarray mix + 1173 μL ddH20 + 102 μL cDNA). From this mixture, 25 μL were added to each well of a 96-well plate of the PCR array [16].

cDNA Synthesis and Quantitative RT-PCR Analysis

Using a cDNA synthesis kit (Applied Biosystems, Foster City, California, USA), cDNA was synthesized from 1.0 μg of total RNA. For the RT-PCR analysis (Stratagene, California, TX, USA), 1.0 μg of cDNA per 25 μL final reaction volume was used. PCR reactions were performed using

Table 1 Functional gene grouping, symbols and the description of the genes in mouse osteogenesis PCR array

Functional gene groupings	GenBank	Symbol	Description
Skeletal development/bone mineralization/occification Extracellular matrix proteins (ECM)/ECM protease inhibitors	NM_013465	Ahsg	Alpha-2-HS-glycoprotein
Extracellular matrix (ECM) proteins/ECM molecules	NM_007431	Akp2	Alkaline phosphatase 2, liver
Skeletal development/bone mineralization/occification Extracellular matrix proteins/structural constituents of tooth enamel	NM_009664	Ambn	Ameloblastin
Bone mineral metabolism/calcium ion binding-homeostasis	NM_009673	Anxa5	Annexin A5
Extracellular matrix (ECM) proteins/ECM molecules	NM_007542	Bgn	Biglycan
Bone mineral metabolism/calcium ion binding-homeostasis Cell growth-differentiation/growth factors-receptors	NM_009755	Bmp1	Bone morphogenetic protein 1
Extracellular matrix proteins/ECM proteases			
Skeletal development genes Cell growth-differentiation/growth factors-receptors/cell differentiation	NM_007553	Bmp2	Bone morphogenetic protein 2
Cell growth-differentiation/growth factors-receptors	NM_173404	Bmp3	Bone morphogenetic protein 3
Skeletal development genes Cell growth-differentiation/growth factors-receptors/cell differentiation	NM_007554	Bmp4	Bone morphogenetic protein 4
Skeletal development genes bone mineral metabolism/phosphate transport Cell growth-differentiation/growth factors-receptors	NM_007555	Bmp5	Bone morphogenetic protein 5
Skeletal development genes Cell growth-differentiation/growth factors-receptors/cell differentiation	NM_007556	Bmp6	Bone morphogenetic protein 6
Cell growth-differentiation/growth factors-receptors	NM_009758	Bmpr1a	Bone morphogenetic protein receptor, type 1A
Skeletal development/cartilage condensation Cell growth-differentiation/growth factors-receptors	NM_007560	Bmpr1b	Bone morphogenetic protein receptor, type 1B
Cell adhesion molecules	NM_007643	Cd36	CD36 antigen
Bone Mineral Metabolism/calcium ion binding-homeostasis Cell adhesion molecules/cell-cell adhesion	NM_009866	Cdh11	Cadherin 11
Bone mineral metabolism/phosphate transport	NM_009925	Col10a1	Procollagen, type X, alpha 1
Skeletal development/cartilage condensation Bone mineral metabolism/phosphate transport	NM_007729	Col11a1	Procollagen, type XI, alpha 1
Extracellular matrix proteins/collagens			
Cell adhesion molecules			
Bone mineral metabolism/phosphate transport Cell adhesion molecules	NM_007730	Col12a1	Procollagen, type XII, alpha 1
Bone mineral metabolism/phosphate transport Cell adhesion molecules	NM_181277	Col14a1	Procollagen, type XIV, alpha 1
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/collagens	NM_007742	Col1a1	Procollagen, type I, alpha 1
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/collagens	NM_007743	Col1a2	Procollagen, type I, alpha 2
Skeletal development/cartilage condensation Bone mineral metabolism/phosphate transport	NM_031163	Col2a1	Procollagen, type II, alpha 1
Extracellular matrix proteins/collagens			
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/collagens	NM_009930	Col3a1	Procollagen, type III, alpha 1
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/basement membrane constituents	NM_009931	Col4a1	Procollagen, type IV, alpha 1
Extracellular matrix proteins/collagens			
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/basement membrane constituents	NM_009932	Col4a2	Procollagen, type IV, alpha 2
Extracellular matrix proteins/collagens			

Table 1 (continued)

Functional gene groupings	GenBank	Symbol	Description
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/collagens	NM_015734	Col5a1	Procollagen, type V, alpha 1
Cell adhesion molecules			
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/collagens	NM_009933	Col6a1	Procollagen, type VI, alpha 1
Cell adhesion molecules			
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/collagens	NM_146007	Col6a2	Procollagen, type VI, alpha 2
Cell adhesion molecules			
Bone mineral metabolism/phosphate transport Extracellular matrix proteins/ECM protease inhibitors	NM_007738	Col7a1	Procollagen, type VII, alpha 1
Bone mineral metabolism/calcium ion binding-homeostasis Cell adhesion molecules	NM_016685	Comp	Cartilage oligomeric matrix protein
Cell growth-differentiation/growth factors-receptors/cell differentiation	NM_009969	Csf2	Colony stimulating factor 2 (granulocyte-macrophage)
Cell growth-differentiation/growth factors-receptors	NM_009971	Csf3	Colony stimulating factor 3 (granulocyte)
Extracellular matrix proteins/ecm proteases	NM_007802	Ctsk	Cathepsin K
Skeletal development/occification	NM_016779	Dmp1	Dentin matrix protein 1
Bone mineral metabolism/calcium ion binding-homeostasis Cell growth-differentiation/growth factors-receptors	NM_010113	Egf	Epidermal growth factor
Skeletal development/bone mineralization/occification Extracellular matrix proteins/structural constituents of tooth enamel	NM_017468	Enam	Enamelin
Cell growth-differentiation/regulation of cell cycle/cell proliferation Cell growth-differentiation/growth factors-receptors	NM_010197	Fgf1	Fibroblast growth factor 1
Cell growth-differentiation/regulation of cell cycle/cell proliferation Cell growth-differentiation/growth factors-receptors/cell differentiation	NM_008006	Fgf2	Fibroblast growth factor 2
Cell growth-differentiation/regulation of cell cycle/cell proliferation Cell growth-differentiation/growth factors-receptors	NM_008007	Fgf3	Fibroblast growth factor 3
Cell growth and differentiation/growth factors-receptors	NM_010206	Fgfr1	Fibroblast growth factor receptor 1
Skeletal development/bone mineralization Cell growth-differentiation/cell proliferation	NM_010207	Fgfr2	Fibroblast growth factor receptor 2
Cell growth-differentiation/growth factors-receptors	NM_010228	Flt1	FMS-like tyrosine kinase 1
Cell adhesion molecules	NM_010233	Fn1	Fibronectin 1
Cell growth-differentiation/growth factors-receptors	NM_145741	Gdf10	Growth differentiation factor 10
Cell adhesion molecules/cell-cell adhesion	NM_010493	Icam1	Intercellular adhesion molecule
Cell growth-differentiation/growth factors-receptors/cell differentiation	NM_010512	Igf1	Insulin-like growth factor 1
Cell growth-differentiation/growth factors-receptors	NM_010513	Igf1r	Insulin-like growth factor I receptor
Cell adhesion molecules/cell-matrix adhesion	NM_008396	Itga2	Integrin alpha 2
Cell adhesion molecules/cell-matrix adhesion	NM_010575	Itga2b	Integrin alpha 2b
Cell adhesion molecules/cell-matrix adhesion	NM_013565	Itga3	Integrin alpha 3
Cell adhesion molecules/cell-matrix adhesion	NM_008401	Itgam	Integrin alpha M
Cell adhesion molecules/cell-matrix adhesion	NM_008402	Itgav	Integrin alpha V
Cell growth-differentiation/regulation of cell cycle Cell adhesion molecules/cell-matrix adhesion	NM_010578	Itgb1	Integrin beta 1 (fibronectin receptor beta)
Extracellular matrix proteins/ECM proteases	NM_019471	Mmp10	Matrix metallopeptidase 10
Bone mineral metabolism/calcium ion binding and homeostasis Extracellular matrix proteins/ECM proteases	NM_008610	Mmp2	Matrix metallopeptidase 2
Bone mineral metabolism/calcium ion binding and homeostasis Extracellular matrix proteins/ecm proteases	NM_008611	Mmp8	Matrix metallopeptidase 8
Extracellular matrix proteins/ECM proteases	NM_013599	Mmp9	Matrix metallopeptidase 9

Table 1 (continued)

Functional gene groupings	GenBank	Symbol	Description
Cell adhesion molecules/transcription factors-regulators	NM_010835	Msx1	Homeo box, msh-like 1
Cell adhesion molecules/transcription factors-regulators	NM_008689	Nfkb1	Nuclear factor of kappa light chain gene enhancer in B-cells 1, p105
Cell growth-differentiation/regulation of cell cycle/cell proliferation/growth factors-receptors	NM_008808	Pdgfa	Platelet derived growth factor, alpha
Skeletal development/occification	NM_011077	Phex	Phosphate regulating gene with homologies to endopeptidases
Extracellular matrix proteins/ECM proteases			on the X chromosome (hypophosphatemia, vitamin D resistant rickets)
Skeletal development genes Cell growth-differentiation/cell differentiation	NM_009820	Runx2	Runt related transcription factor 2
Cell adhesion molecules/transcription factors-regulators			
Cell growth-differentiation/growth factors-receptors Cell adhesion molecules	NM_016741	Scarb1	Scavenger receptor class B, member 1
Extracellular matrix proteins/ECM protease inhibitors	NM_009825	Serpinh1	Serine (or cysteine) peptidase inhibitor, clade H, member 1
Skeletal development/bone mineralization Cell adhesion molecules/transcription factors-regulators	NM_008539	Smad1	MAD homolog 1 (Drosophila)
Cell growth-differentiation/cell differentiation Cell adhesion molecules/transcription factors-regulators	NM_010754	Smad2	MAD homolog 2 (Drosophila)
Cell growth-differentiation/cell proliferation Cell adhesion molecules/transcription factors-regulators	NM_016769	Smad3	MAD homolog 3 (Drosophila)
Cell adhesion molecules/transcription factors-regulators	NM_008540	Smad4	MAD homolog 4 (Drosophila)
Skeletal development/occification	NM_024449	Sost	Sclerostin
Skeletal development/cartilage condensation Cell growth-differentiation/cell differentiation	NM_011448	Sox9	SRY-box containing gene 9
Cell adhesion molecules/transcription factors-regulators			
Skeletal development/occification Cell growth-differentiation/cell differentiation	NM_018783	Tfip11	Tuftelin interacting protein 11
Skeletal development genes Cell growth-differentiation/regulation of cell cycle/cell proliferation/growth factors-receptors	NM_011577	Tgfb1	Transforming growth factor, beta 1
Cell growth-differentiation/regulation of cell cycle/cell proliferation/growth factors-receptors	NM_009367	Tgfb2	Transforming growth factor, beta 2
Cell growth-differentiation/regulation of cell cycle/cell proliferation/growth factors-receptors	NM_009368	Tgfb3	Transforming growth factor, beta 3
Cell growth-differentiation/growth factors-receptors	NM_009370	Tgfbr1	Transforming growth factor, beta receptor I
Cell growth-differentiation/cell proliferation/growth factors-receptors/cell differentiation	NM_009371	Tgfbr2	Transforming growth factor, beta receptor II
Cell growth-differentiation/growth factors-receptors	NM_011578	Tgfbr3	Transforming growth factor, beta receptor III
Skeletal development/osteoclast differentiation	NM_013693	Tnf	Tumor necrosis factor
Skeletal development/bone mineralization/occification Extracellular matrix proteins/structural constituents of tooth enamel	NM_011656	Tuft1	Tuftelin 1
Cell growth-differentiation/cell differentiation Cell adhesion molecules/transcription factors-regulators	NM_011658	Twist1	Twist gene homolog 1 (Drosophila)
Cell adhesion molecules/cell-cell adhesion	NM_011693	Vcam1	Vascular cell adhesion molecule 1
Skeletal development genes/calcium ion binding-homeostasis Cell growth-differentiation/growth factors-receptors	NM_009504	Vdr	Vitamin D receptor
Cell adhesion molecules/transcription factors-regulators			
Cell growth-differentiation/regulation of cell cycle/cell proliferation/growth factors-receptors	NM_009505	Vegfa	Vascular endothelial growth factor A
Cell growth-differentiation/regulation of cell cycle/cell proliferation	NM_011697	Vegfb	Vascular endothelial growth factor B

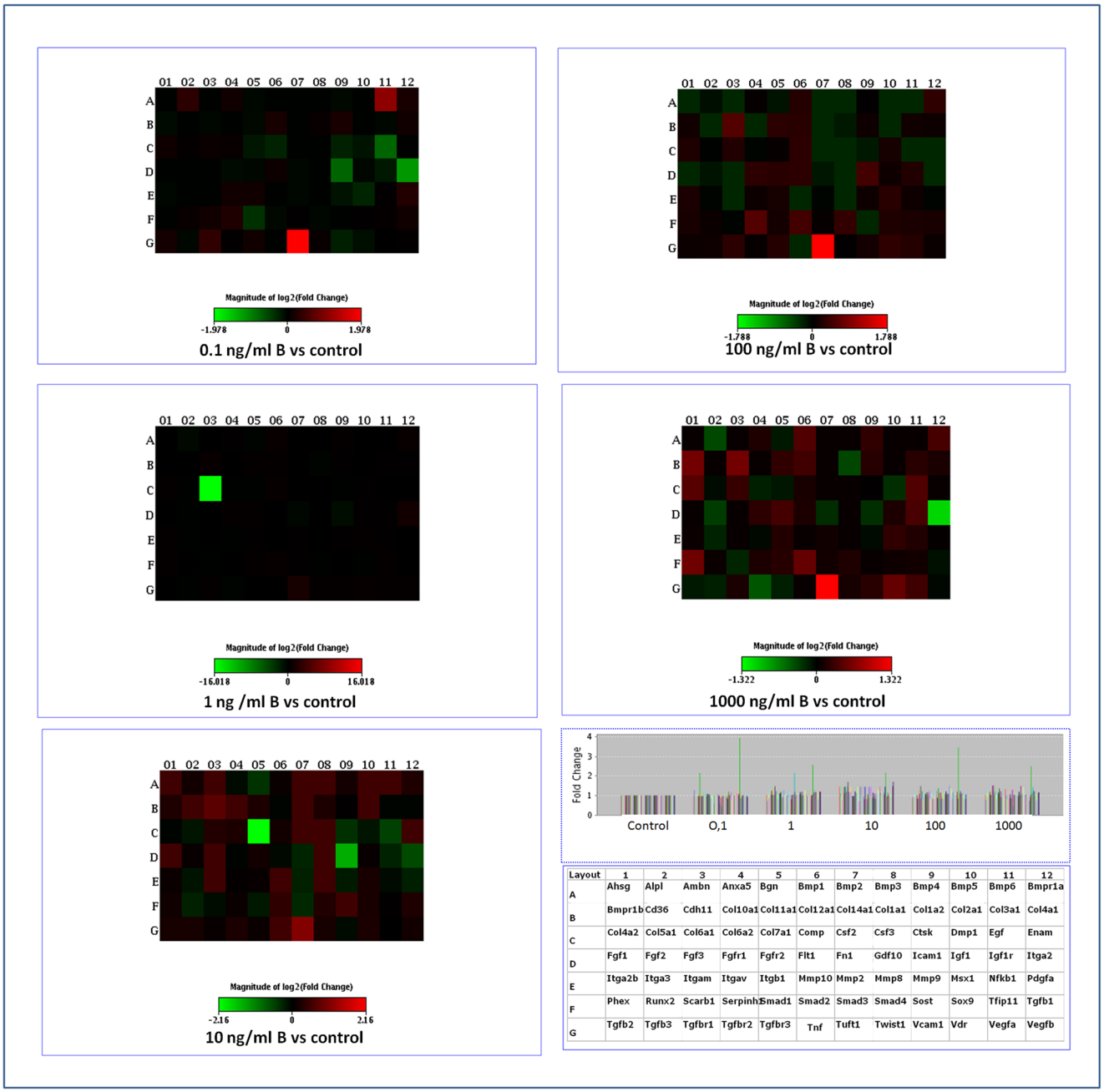

Fig. 1 Comparison and fold changes of the genes diagram of genes associated with osteogenesis of MC3T3-E1 cells with different boric acid concentrations (0.1, 1, 10, 100, and 1000 ng/ml). Please note the layout of the PCR array (bottom right)

the Tuftelin1 universal library probe (04688112001-Roche). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a housekeeping/reference gene for normalization. The amplification profile for the tuftelin and GAPDH was 95 °C for 10 min followed by 40 cycles of 95 °C/15 s and 60 °C/1 min using Stratagene MX3000P.

Statistical Analysis

For PCR array data analysis; web portal was used for calculations (tabular format, a scatter plot, a three-dimensional profile, and a volcano plot) and interpretation of the control wells upon including threshold cycle data from a real-time instrument.

For mRNA expression analysis, one-way analysis of variance and Tukey HSD (honestly significant difference) multiple comparison tests were used to compare groups (GraphPad Software, San Diego, CA). The data are presented as mean ± standard deviation. A value of $p < 0.05$ was considered statistically significant, and a value of $p < 0.01$ was considered statistically highly significant.

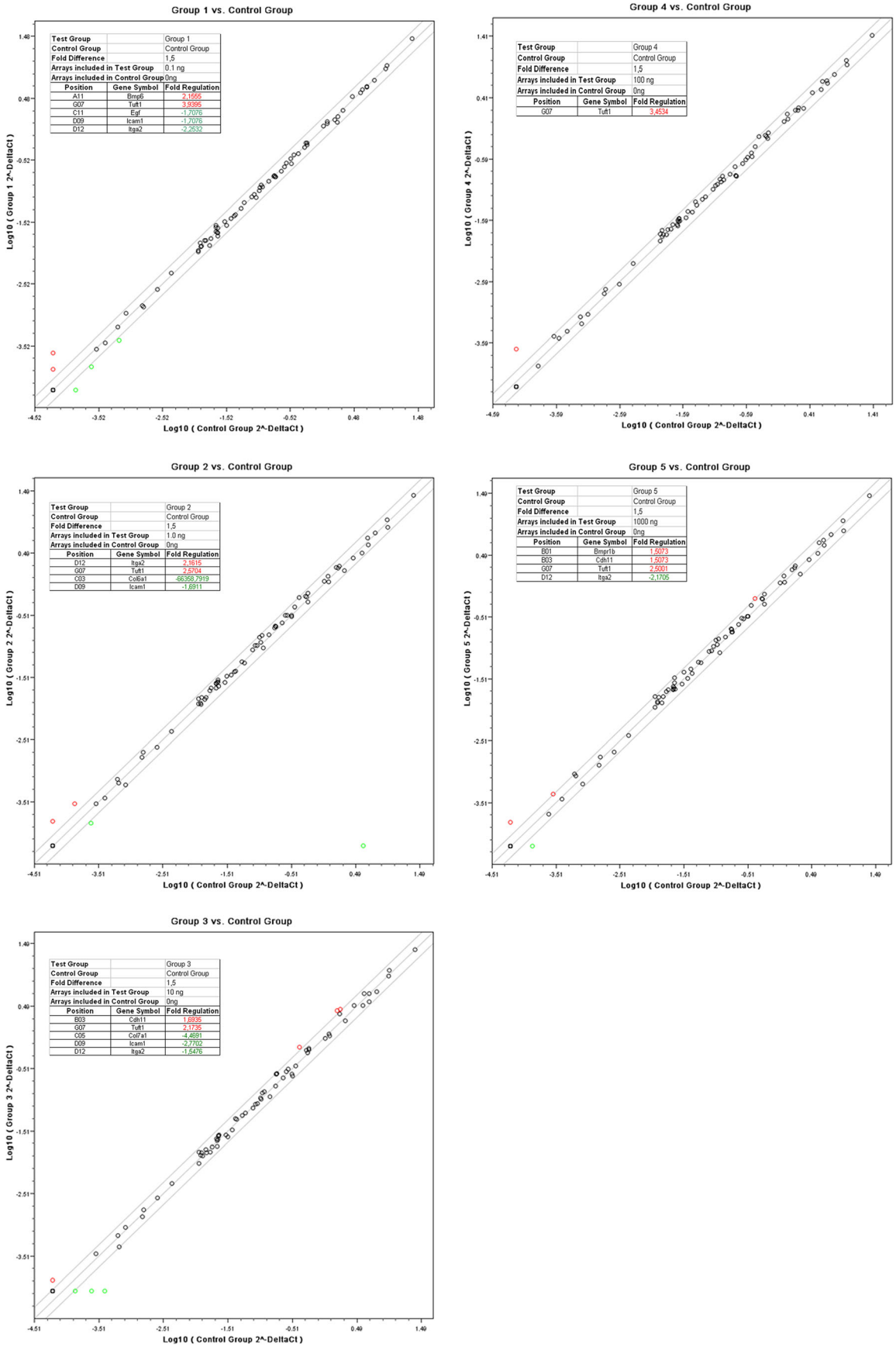

Fig. 2 Significant up and downregulated genes of MC3T3-E1 cells exposed to different concentrations of boric acid

Results

As indicated by Figs. 1 and 2, all boron as boric acid treatments compared with controls upregulated tuftelin mRNA. In addition to tuftelin, the boric acid treatments modulated integrin α2 (Itga2), and intercellular adhesion molecules (ICAM) mRNA expressions. The 0.1, 1, 10, and 1000 ng/mL treatments upregulated Itga2 mRNA expression which is involved in cell adhesion and cell-surface-mediated signaling. While 100 ng/ml has no effect on the Itga2 transcript and 1 ng/ml boric acid upregulated Itga2 mRNA expression (2.1-fold), 0.1, 10, and 1000 ng/ml boric acid downregulated the Itga2 gene transcript 2.2-, 1.5-, and 2.1-fold, respectively. The 0.1, 1, and 10 ng/ml boric acid treatments decreased ICAM mRNA expression, which is involved inflammation, immune response, and intracellular signaling. In addition to tuftelin, Itga2, and ICAM mRNA expression, other changed expressions were inconsistently found. While 0.1 ng/ml boric acid induced BMP6 (2.1-fold), 1000 ng/ml boric acid treatment increased BMP1r mRNA expression (1.5-fold). Decreased Epidermal Growth Factor mRNA expression (1.7-fold) was observed in 0.1 ng/ml boric acid group. While 1.0 ng/ml boric acid treatment increased Col61a, 10 ng mL boric acid treatment decreased Col7a1 mRNA expressions (4.4-fold) (Figs. 1 and 2).

The quantitative RT-PCR results shown in Fig. 3 confirmed the PCR-array tuftelin findings. All boric acid treatments except the 1000 ng/ml treatment statistically significantly increased tuftelin mRNA expression.

Discussion

The present findings are consistent with previous studies showing that boron promotes bone formation and remodeling through modulating genes involved in osteogenesis. The upregulation of tuftelin by boron indicates that boron has its effect in the initial stages of bone and tooth formation and mineralization. In embryonic development, tuftelin mRNA was already expressed in the E10.5 stage before the initiation of tooth development [13]. At the E15 stage when alveolar bone and vasculation appears, tuftelin was histologically seen in osteoblasts [12]. Tuftelin is abundant in the cytoplasm of differentiating osteoblasts, which suggests that tuftelin has a critical role in the differentiation of mesenchymal cells into osteoblasts. The increase in tuftelin mRNA in pre-osteoblastic MC3T3-E1 cells by boron suggests that a major influence of boron on bone and tooth formation and mineralization is through genetically enhancing the differentiation or formation of osteoblasts. This suggestion is supported by the findings that boron deprivation depresses the number of osteoblasts in alveolar bone repair [3, 4]. Enhancing the development of pre-osteoblasts into osteoblasts might be a basis for boron increasing mRNA expression and amounts of osteocalcin, bone sialoprotein, type 1 collagen, alkaline phosphatase, and bone morphogenetic proteins in MC3T3-E1 cells [1]. Osteoblast differentiation also might be the basis for some of the significant changes found for other osteogenetic expressions found in the present study.

An effect on tuftelin mRNA expression also might be a basis for apparently essential actions of boron. Tuftelin is present in morula and embryonic stem cells [17, 18] and found in other soft organs including lung, liver, kidney and testis [19, 20]. These findings suggest that tuftelin is critical for embryo development. Boron has been described as essential for embryo development of zebrafish [21] and the African clawed frog [22].

Similar to MC3T3-E1 cells, physiological amounts of boron has been found to modulate gene expression in DU-145 prostrate cells [15]. In these cells, boron activated transcription factors involved in the induction anti-inflammatory genes, the differentiation of osteoblasts, the mineralization of teeth, tumor suppression, and retinal degeneration prevention. All these are beneficial actions for which boron has been reported [23]. Boric acid activation of genes may be the basis for wide range of diverse phenotypic responses associated with boron deprivation or supplementation.

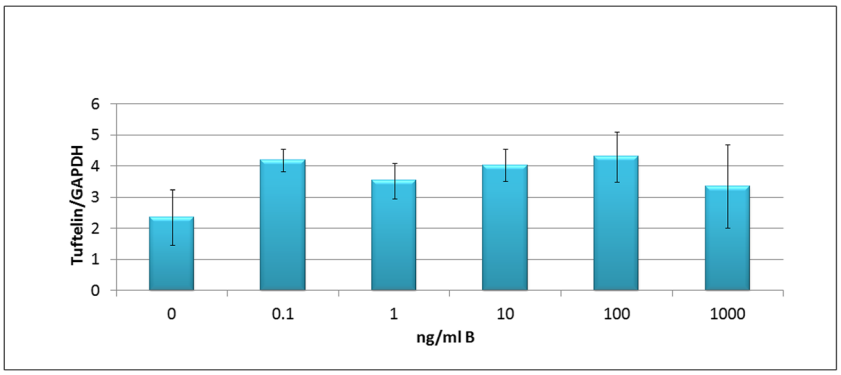

Fig. 3 Tuftelin mRNA expression of MC3T3-E1 cells treated with 0.1, 1, 10, 100 and 1000 ng/ml boric acid. Significant increase was observed in 0.1, 1, 10, and 100 ng/ml boric acid treated groups when compared with untreated control group ($p < 0.05$)

The mechanism through which boric acid induces gene activation has not definitively established. However, the findings with DU-145 prostate cells indicate that boron can modulate intracellular Ca^{2+} concentration involved in the signaling for gene activation [15]. The findings of the present study suggest that boron affects the regulation of the tuftelin1 gene in osteoblastic cells. This effect might be the result of boron influencing signaling pathways, perhaps those mediated by Ca^{2+}. This speculation may explain increased Cd11 as well since it modulates Ca^{2+}-dependent cell-cell adhesion.

Tuftelin is thought to be involved with adaptation to hypoxia, mesenchymal stem cell function, and neurotrophin nerve growth factor-mediated neuronal differentiation. According to our knowledge, this is the first study detecting the importance of boron regulation on the tuft1gene in the osteoblasts. This might have secondary effects on the vicinity of the osteoblasts. Further studies are required to understand the pathway and signaling mechanisms of tuft1 gene. Since this study only evaluated mRNA level, protein levels of Tuft should be also checked. Other cells, i.e., mesenchymal stem cells, should also be evaluated regarding tuft gene to clarify the effects on the osteogenic/neurogenic/adipogenic differentiation.

In a previous study (Hakki et al. 2010), we observed that boron as boric acid increased bone morphogenetic protein (BMP), BMP-4, -6 and -7 protein levels at 0.1, 1, 10, and 100 ng/ml boric acid concentrations [1]. In this experiment, Tuft1 regulation seems definitely more apparent when compared with the other genes related to osteogenesis. Inductive effects of boric acid concentrations below 1000 ng/ml were also the findings of this study. In summary, the upregulation of tuftelin mRNA in pre-osteoblastic MC3T3-E1 cells provides further evidence that nutritional or physiological amounts of boron has beneficial, perhaps critical or essential, effects in animals and humans. This effect is at the molecular level and thus can have numerous beneficial phenotypic effects including being beneficial to alveolar bone and tooth development and mineralization. Since only MC3T3-E1 cell line was used in this study, these finding should be confirmed in other cells like human osteoblasts, primary osteoblastic cells, and mesenchymal stem cells isolated from bone marrow and dental tissues to ensure tuftelin regulation by boric acid and its importance on the mineralized tissues. Further in vivo studies, tuftelin knockout (tuftelin-null mice) animals also could provide more evidence for tuftelin expression in alveolar/other bones and dental tissues during development and postnatal term of the organisms and possible boron interaction.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Hakki SS, Bozkurt BS, Hakki EE (2010) Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1). J Trace Elem Med Biol 24(4):243–250
2. Hakki SS, Dundar N, Kayis SA, Hakki EE, Hamurcu M, Kerimoglu U, Baspinar N, Basoglu A, Nielsen FH (2013) Boron enhances strength and alters mineral composition of bone in rabbits fed a high energy diet. J Trace Elem Med Biol 27(2):148–153
3. Gorustovich AA, Steimetz T, Nielsen FH, Guglielmotti MB (2008) Histomorphometric study of alveolar bone healing in rats fed a boron-deficient diet. Anat Rec (Hoboken) 291(4):441–447
4. Gorustovich AA, Steimetz T, Nielsen FH, Guglielmotti MB (2008) A histomorphometric study of alveolar bone modelling and remodelling in mice fed a boron-deficient diet. Arch Oral Biol 53(7):677–682
5. Seaborn CD, Nielsen FH (1994) Boron and silicon: effects on growth, plasma lipids, urinary cyclic AMP and bone and brain mineral composition in male rats. Environ Toxicol Chem 13:941–947
6. Haro Durand LA, Mesones RV, Nielsen FH, Gorustovich AA (2010) Histomorphometric and microchemical characterization of maturing dental enamel in rats fed a boron-deficient diet. Biol Trace Elem Res 135(1–3):242–252
7. Hakki SS, Malkoc S, Dundar N, Kayis SA, Hakki EE, Hamurcu M, Baspinar N, Basoglu A, Nielsen FH, Götz W (2015) Dietary boron does not affect tooth strength, micro-hardness, and density, but affects tooth mineral composition and alveolar bone mineral density in rabbits fed a high-energy diet, J Trace Elem Med Biol. 29:208–215
8. Deutsch D, Silverstein N, Shilo D, Lecht S, Lazarovici P, Blumenfeld A (2011) Biphasic influence of hypoxia on tuftelin expression in mouse mesenchymal C3H10T1/2 stem cells. Eur J Oral Sci 119(Suppl 1):55–61
9. Alfaqeeh SA, Gaete M, Tucker AS (2013) Interactions of the tooth and bone during development. J Dent Res 92:1129–1135
10. Shilo D, Blumenfeld A, Haze A, Sharon S, Goren K, Hanhan S, Gruenbaum-Cohen Y, Ornoy A, Deutsch D (2019) Tuftelin's involvement in embryonic development. J Exp Zool B Mol Dev Evol 332(5):125–135
11. Shilo D, Cohen G, Blumenfeld A, Goren K, Hanhan S, Sharon S, Haze A, Deutsch D, Lazarovici P (2019b) Tuftelin is required for NGF-induced differentiation of PC12 cells. J Mol Neurosci 68(1):135–143
12. Vesela B, Svandova E, Bobek J, Lesot H, Matalova E (2019) Osteogenic and angiogenic profiles of mandibular bone-forming cells. Front Physiol 10:124
13. Bobek J, Oralova V, Kratochvilova A, Zvackova I, Lesot H, Matalova E (2019) Tuftelin and HIFs expression in osteogenesis. Histochem Cell Biol 152(5):355–363
14. Wang D, Christensen K, Chawla K, Xiao G, Krebsbach PH, Franceschi RT (1999) Isolation and characterization of MC3T3-E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential. J Bone Miner Res 14(6):893–903
15. Kobylewski SE, Hendeson KA, Yamada KE, Eckhert CD (2017) Activation of EIF2α/ATF4 and ATF6 pathways in DU-145 cells by

boric acid at the concentration reported in men at the US mean boron intake. Biol Trace Elem Res 176:278–293
16. Hakki SS, Bozkurt SB, Hakki EE, Korkusuz P, Purali N, Koç N, Timucin M, Ozturk A, Korkusuz F (2012) Osteogenic differentiation of MC3T3-E1 cells on different titanium surfaces. Biomed Mater 7(4):045006
17. Deutsch D, Leiser Y, Shay B, Fermon E, Taylor A, Rosenfeld E, Dafni L, Charuvi K, Cohen Y, Haze A, Fuks A, Mao Z (2002) The human tuftelin gene and the expression of tuftelin in mineralizing and nonmineralizing tissues. Connect Tissue Res 43:425–434
18. Leiser Y, Blumenfeld A, Haze A, Dafni L, Taylor AL, Rosenfeld E, Fermon E, Gruenbaum-Cohen Y, Shay B, Deutsch D (2007) Localization, quantification, and characterization of tuftelin in soft tissues. Anat Rec Adv Integr Anat Evol Biol 290:449–454
19. MacDougall M, Simmons D, Dodds A, Knight C, Luan X, Zeichner-David M, Zhang C, Ryu OH, Qian Q, Simmer JP, Hu CC (1998) Cloning, characterization, and tissue expression pattern of mouse tuftelin cDNA. J Dent Res 77:1970–1978
20. Mao Z, Shay B, Hekmati M, Fermon E, Taylor A, Dafni L, Heikinheimo K, Lustmann J, Fisher LW, Young MF, Deutsch D (2001) The human tuftelin gene: cloning and characterization. Gene 279:181–196
21. Rowe RI, Eckhert CD (1999) Boron is required for zebrafish embryogenesis. J Exp Biol 202:1649–1654
22. Fort DJ, Rogers RL, McLaughlin DW, Sellers CM, Schlekat CL (2002) Impact of boron deficiency on *Xenopus laevis*. A summary of biological effects and potential biochemical roles. Biol Trace Elem Res 90:117–142
23. Nielsen FH, Meacham SL (2011) Growing evidence for human health benefits of boron. J Evid Based Complement Altern Med 16:169–180